Agronomic and physiological performance of four perennial grasses under summer dry conditions in New Zealand

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Abstract of a thesis submitted for a degree of Doctor of Philosophy

By

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Agronomic and physiological performance of four perennial grasses under summer dry conditions in New Zealand

The main aim of this research was to quantify the effects of different levels of moisture and nitrogen (N) supply on dryland brome, cocksfoot, perennial ryegrass and tall fescue pastures grown under summer dry conditions. This was assessed from establishment (2014/15) to the end of the second year (2015/16) in two field experiments.

Moisture and temperature are the major factors that affect germination. To quantify their combined effects, a laboratory experiment was used to calculate hydrothermal time requirements. Increasing temperature from 25 to 30 and 35 °C caused a rapid decline in germination rate of cocksfoot and tall fescue even when moisture was not limited (0 MPa). Applying moisture stress, especially when temperature was higher than 20 °C resulted in a more rapid decline in cocksfoot germination rate and final germination percentage than in perennial ryegrass, tall fescue and brome.

To explore the effects of different soil moisture levels on pasture production in the field, two experiments were established in October 2014 at Ladbrooks and Ashley Dene, which differed in plant available water content (PAWC). The Wakanui soil at Ladbrooks has a 41-55% higher PAWC than the stony Lismore silt soil at Ashley Dene. A Latin square design was established at both sites with four grass species and four replicates. During establishment period, from October 2014 to June 2015, total dry matter (DM) production at Ladbrooks was 4.34 t ha^{-1} compared with 1.93 t ha^{-1} at Ashley Dene, with no differences among species.

In the second year from July 2015 to July 2016, the experiments included a nitrogen (N) treatment. At Ladbrooks 900 kg N ha^{-1} and at Ashley Dene 500 kg N ha^{-1} were applied. In 2015/16, mean annual DM production was 19.8 t ha^{-1} for +N and 9.20 t ha^{-1} for −N treatments at Ladbrooks. At this location, there were no differences for DM production among the species. In 2015/16 at Ashley Dene, the +N cocksfoot treatment DM was 6.0 t ha^{-1} higher than the average of 3.2 t ha^{-1} for the other ±N species. This difference at Ashley Dene was explained by the interaction between species and N after 85 mm of rainfall in mid-January of 2016, when only the cocksfoot responded to the applied N.

The impact of seasonal temperatures on DM yield was quantified using thermal time accumulated above a base temperature of 3 °C. In the 2015/16 period, the relationship between accumulated

thermal time and DM was separated into two phases at Ladbrooks and into five phases at Ashley Dene. These phases indicated decreased or increased growth due to the onset of moisture stress or rainfall. At Ladbrooks, the break points were not different among species, but were later in +N compared with –N treatments. For +N pastures linear growth in Phase 1 lasted until 6/3/2016, after 2355 ± 44.6 °Cd. For −N treatments Phase 1 stopped after only 2161 °Cd, on 19/2/2016. The temperature adjusted linear growth rate was differed among species and N treatments. For cocksfoot and perennial ryegrass it was 6.94 kg DM °Cd^{-1} ha^{-1} which was higher than brome and tall fescue (6.00 kg DM °Cd^{-1} ha^{-1}). At Ashley Dene, the additional yield from +N cocksfoot resulted in a higher linear growth rate in Phase 3, after the 85 mm of rainfall in January 2016.

Plant available water content was 254 mm for +N and 195 mm for −N pastures at Ladbrooks compared with a mean of 101 mm at Ashley Dene. In the 2015/16 period, mean total water use was 823 mm for +N pastures which was higher than the −N pastures (777 mm) at Ladbrooks. There were no differences among species. Mean water use efficiency for +N pastures was 20.4 kg $ha^{-1}mm^{-1}$ which was higher than −N pastures (8.36 kg $ha^{-1}mm^{-1}$). There were no differences among the species. In 2015/16, total water use at Ashley Dene was 364 mm and was not different among species. However, water use efficiency for cocksfoot was 16.6 kg $ha^{-1}mm^{-1}$ which was higher than other species.

The higher production of + pastures at Ladbrooks was explained by higher radiation interception and also radiation use efficiency (RUE). The lower yield at Ashley Dene compared with Ladbrooks was due to the reduction in the amount of photosynthetically active radiation intercepted by the canopies as well as a lower RUE. Higher radiation interception of +N cocksfoot on 20/1/2016 and 21/4/2016 was the main explanation for its higher DM production.

Overall, the range of dry matter production yield from the different soil moisture and N conditions could be quantified by calculation of growth rates in relation to thermal time, soil water extraction patterns, estimation of light interception and radiation use efficiency, explain the physiological basis of yield differences.

Key words: brome (*Bromus valdivianus* Phil.), cocksfoot (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.), tall fescue (*Festuca arundinacea* Schreb.; syn. *Schedonorus phoenix* (Scop.))

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Chapter 1 General Introduction

1.1 The economic cost of drought

Pastoral agricultural production is significantly affected by climate variability. This has a major impact on the economy. For example, during the 1997/98 El Niño drought, the estimated reduction of agriculture (pastoral sector) farm-gate income exceeded $425 million. In 2012/13, large areas of New Zealand's North Island experienced drought resulting in significant financial hardship for many farmers.

Reducing the economic cost of drought to the agricultural sector requires the assessment of plants which are more tolerant of drought than those currently used. This will reduce agricultural vulnerability and secure livelihoods of those who depend on agricultural income.

1.2 Summer dry periods in New Zealand

Currently summer droughts may last three to four months in east coast regions of New Zealand's South Island. However, climate change scenarios suggest the duration and intensify of drought may increase in the next few decades (Moot *et al*., 2010). Temperatures in New Zealand are moderated by the South Pacific and Antarctic Oceans. Thus, it is predicted to experience only about 70% of the average global temperature change anticipated over the next 10 years. This is mainly because, the temperature in New Zealand is under control of the South Pacific and Antarctic Oceans, which are predicted to be affected only gradually by global temperature changes (Kenny, 2001). Some regions, such as Southland, are expected to experience warmer winters and a longer growing season. However, variability in summer rainfall is already an issue for dryland regions of New Zealand and is expected to increase (Salinger, 2003). The difference in average precipitation between western and eastern areas of New Zealand is estimated to become greater and rainfall is likely to rise in the west of the country and decline in the east. A decrease in precipitation is expected in eastern areas of Gisborne, Hawke's Bay, Wairarapa, Marlborough and Canterbury and be followed by increases in temperature and decreased run-off into rivers through greater soil evaporation. Thus, these regions are predicted to experience more regular and severe droughts, and in non-irrigated down-land regions some river reaches could dry up, leading to greater pressure on current water resources (Moot *et al*., 2009). Currently, on the east coast, summer soil moisture deficits usually lead to no pasture production in dryland agricultural systems. This constrains growth rates more than temperature does in the winter. Selecting more drought tolerant forage crops could mitigate some current and anticipated impacts of climate changes.

1.3 Pasture production in summer dry areas

In New Zealand, most non-irrigated dairy farms are located in 'summer safe' regions including Taranaki, in the North Island and Southland and the West Coast of the South Island. These have suitable conditions for pasture growth, with annual average precipitation greater than 1500 mm. Periods of moisture stress are short and rare. Because returns from milk have been higher than from meat and wool in recent years, dairying has expanded into traditional sheep and beef areas on the east coast of the South Island where irrigation is available. These systems may require up to 450 mm of irrigation water to overcome soil moisture deficits in these traditionally summer dry regions. The availability of irrigation has led to rapid and extensive use in Canterbury over the last two decades (Moot *et al*., 2010. Less than 3% of New Zealand's entire land area (~620 000 ha) is under irrigation that includes horticulture, floriculture, viticulture, agriculture and forestry industries. About 84% of this irrigated land is in the South Island, predominantly in Canterbury by default (Department of Statistics, 2002).

Pasture production in low annual rainfall areas such as Canterbury, is usually unaffected by soil moisture deficit in winter and early spring. However, from September to April, soil moisture deficits are common during which more water is lost from soil through evapotranspiration than is supplied by rainfall (Porteous *et al*., 1994). The difference between potential evapotranspiration and precipitation in a growing season (July-June) is called the potential soil moisture deficit. Grass based pasture production decreases when the potential soil moisture deficits surpasses 100 mm and a significant reduction occurs when moisture deficits exceed 150 mm (McAneney *et al*., 1983). Total accumulated potential soil moisture deficits through the growing season normally reach 300–500 mm yr^{-1} in eastern New Zealand. By the beginning of summer, it reaches 100 mm in most years after which a significant growth reduction occurs (Salinger, 2003).

Herbage production is also affected by soil type (texture, proportion of stones, soil depth to stones or rock), the ability of plants to extract water (rooting depth, timing of maximum seasonal demand) and local differences in precipitations. These factors are not easy to manipulate. Thus, appropriate pasture management and identifying suitable and productive dryland species are essential to maintain viable sheep and beef farming in dryland regions of New Zealand. Understanding pasture responses to moisture stress is an important step into develop appropriate management packages for their introduction into farm systems.

The research in this thesis focused on seasonal production of four perennial pasture grasses under moisture-limited conditions of dryland pastures in New Zealand. Differences in their agronomic and physiological performance are quantified. Factors included their ability to extract water and respond to N application from sowing through the first 18 months of their establishment phase. Grasses able to tolerate summer moisture deficits and maintain productivity are identified.

1.4 Aim, objectives and thesis structure

The main aim of this research was to quantify, moisture and N effects on dryland brome (*Bromus valdivianus* Phil.), cocksfoot (*Dactylis glomerata* L.), perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.; syn. *Schedonorus phoenix* Scop.) growth in pastures under summer dry conditions. To create different soil moisture regimes, two field experiments were conducted in the same climate, but on two sites (Ladbrooks and Ashley Dene) which differ in water holding capacity. Experimental sites were established in 2014/15. In the subsequent year (2015/16), N fertiliser was applied to 16 (+N) plots for comparison with 16 control (−N) plots at each site. This study reports on results of the first 18 months. Ongoing measurements beyond those reported in this thesis will allow further interpretation of the dataset.

The thesis is composed of seven chapters (Figure 1-1). Chapter 1 is the introduction. Chapter 2, is a review of the literature with emphasis on the agronomic and physiological reasons for yield differences among temperate perennial pasture grasses. Techniques used to define the influences of environmental variables in this thesis are also reviewed.

Chapter 3 describes germination studies evaluating the combined effects of different levels of moisture and temperature on germination of brome, cocksfoot, perennial ryegrass and tall fescue.

Yield and quality are addressed with the field studies described in Chapter 4. Chapter 5 addresses moisture stress. In Chapter 6 canopy expansion is measured (quantified by LAI) and light interception is calculated to determine their influence on dry matter production. Chapter 7 summarizes the main findings of the research and suggests future research topics and projects.

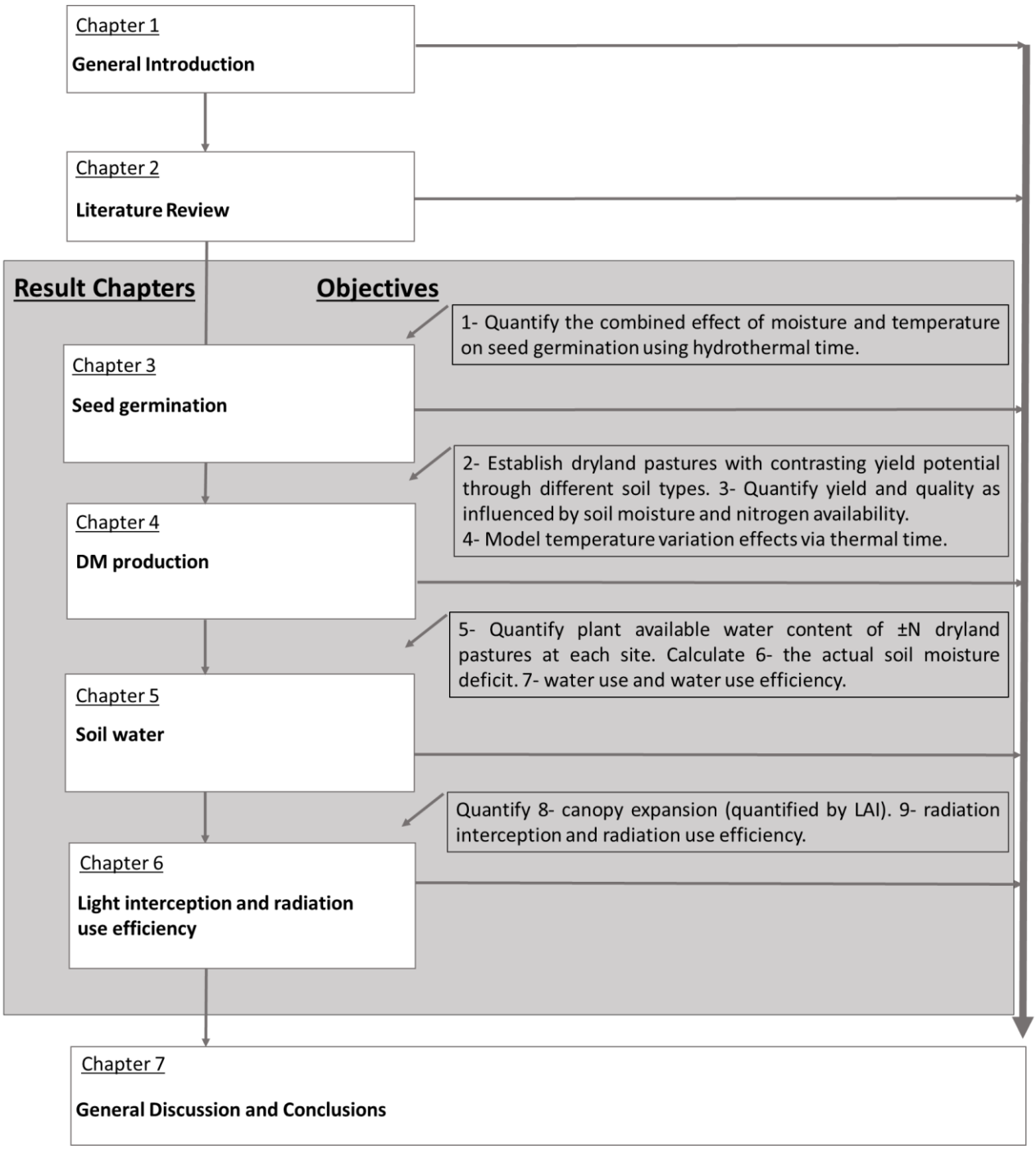

Figure 1-1 Diagram of thesis structure and objectives

Chapter 2 Literature review

Dryland pastoral regions in New Zealand are frequently subjected to periods of summer/autumn moisture stress. This decreases yield, persistence and recovery of traditional perennial ryegrass/white clover (*Trifolium repens* L.) pastures (Woodman *et al*., 1992; Knowles *et al*., 2003). Under these conditions, inclusion of more drought tolerant grasses and legume combinations improves pasture production (Brown *et al*., 2006).

There are a range of adaptations to moisture stress at different levels among pasture species (Xoconostle-Cazares *et al*., 2010). The current research made crop based measurements as required to assess which strategies were being utilised by each species to explain their responses to moisture stress. Intercepted light by a crop/pasture and the efficiency with which energy is utilized are the most important factors that affect crop/pasture production (Monteith, 1972; Biscoe and Gallagher, 1977; Monteith, 1977). The literature reviewed in this chapter is focused on the mechanisms and factors that contribute to pasture yield formation. The review outlines the major attributes of four cool-season (C3) pasture grass species (brome, cocksfoot, perennial ryegrass and tall fescue) used in New Zealand and summarises their reported response to moisture deficit and N.

Establishment is the first step that affects pasture production and can be divided into two phases: seed germination and seedling emergence, followed by seedling growth and plant survival (Brougham 1969). As a part of current research, germination/emergence was measured for brome, cocksfoot, perennial ryegrass and tall fescue. In this chapter previous studies regarding major factors influencing germination/emergence will also be reviewed.

2.1 Yield formation components and effect of nitrogen

Yield formation is a function of incident photosynthetically active radiation (PAR), the fraction of PAR intercepted by the canopy (R/R0) and the radiation use efficiency (RUE) of the transformation of PAR to DM (Equation 2-1). In Equation 2-1, HI is the harvest index which is the quantity of utilised herbage in the form of fruit, grain or fibre which has an economic value. HI is not typically considered for pastures because DM is consumed or harvested (Thornley, 1998).

Equation 2-1 $\text{Yield} = \text{Ro} * \text{R/Ro} * \text{RUE} * \text{HI}$

Intercepted PAR by the canopy depends on leaf expansion, leaf appearance rate (LAR), tillering propensity and canopy architecture (Biscoe and Gallagher, 1977; Hay and Walker, 1989). Therefore, any factor that reduces these will limit yield. In this regard, some environmentally mediated variables (like temperature) and stress conditions alter crop/pasture production (Monteith, 1969, 1972; Biscoe

and Gallagher, 1977; Monteith, 1977; Hay and Walker, 1989). As an example, canopy expansion is restricted by moisture stress which causes a reduction in the amount of light intercepted (Hsiao, 1973; Belaygue *et al*., 1996).

Nitrogen supply affects plant growth and productivity by changing leaf area and photosynthetic capacity (Novoa and Loomis, 1981). Previous studies have suggested that N deficiency limits growth mainly by limiting the rate of leaf area increase (e.g. Watson, 1952), while others have suggested that, growth limitation by low N results mainly from a decreased photosynthetic rate (e.g. Bolton and Brown, 1980). Muchow and Davis (1988) showed that, both radiation interception and RUE were affected by N deficiency in maize (*Zea mays* L.) and sorghum (*Sorghum bicolour* L.).

Radiation use efficiency is an indirect aggregate of net photosynthesis and shows the efficiency with which a pasture consumes intercepted light energy and transforms it to dry matter (Monteith, 1972, 1977; Sinclair and Muchow, 1999a). More than 50% of soluble plant N is directly related to formation of the photosynthetic system (Horst and Nelson, 1979; Sinclair and Horie, 1989). Therefore, RUE is closely related to the N status of the pasture. N is necessary in protein development and formation of nucleic acids, chlorophyll and Ribulose 1,5 biphosphatecarboxylase (Rubisco) (Novoa and Loomis, 1981). In C3 plants, Rubisco accounts for more than 50% of the soluble protein (Schmitt and Edwards, 1981).

Current literature is reviewed in this chapter to understand physiological responses of pastures to moisture, N and temperature and their relation to light interception and radiation use efficiency. Emphasis is focused on the canopy level rather than a single plant or cellular level.

2.2 Temperature impacts

Maximum potential yield is closely related to the environment in which the plants are grown (Mitchell, 1963; Monteith, 1972). Management factors such as duration of regrowth period and environmental elements (soil moisture, temperature, N and solar radiation) have the greatest impact on yield differences among years, seasons and environments (Thornley, 1998). Under non-limiting moisture conditions, temperature is the main factor that affects the rate of canopy expansion (Radcliffe and Baars, 1987). Baars and Waller (1979) showed a mean daily growth rate of 5 kg DM ha^{-1} d^{-1} in winter compared with 49 kg DM ha^{-1} d^{-1} in summer in irrigated perennial ryegrass at Winchmore, in Canterbury.

In a pasture, the plant canopy is periodically reduced by grazing, so recovery from grazing, particularly leaf expansion to re-establish green leaf area in the canopy, is the primary factor that affects yield. Temperature also affects this canopy expansion rate and RUE (Biscoe and Gallagher, 1977).

2.2.1 Quantifying temperature, thermal time (Tt)

Thermal time (Tt) is commonly calculated to quantify the effect of temperature on plant development processes (germination, emergence, leaf appearance) (Baars and Waller, 1979; Jamieson *et al*., 1998a; Moot *et al*., 2000; Mills *et al*., 2006).

Thermal time is accumulated on a daily basis from the mean hourly temperature using Equation 2-2:

Equation 2-2 $\mathrm{Ttdaily}\ (°\mathrm{Cd}) = \Sigma\ (\mathrm{Th} - \mathrm{Tb})$

In which Th is hourly air temperature and the sum of (Th – Tb) provides daily thermal time accumulation (Ttdaily). These are then summed to calculate accumulated Tt for a specific regrowth cycle.

Thermal time calculations can be used to explain seasonal variations and its impact on pasture growth rates. This allows extrapolation of phenology and developmental status results to other environments. Thermal time calculations have been used frequently in simulation models, mainly to predict canopy development, flowering and annual crop maturity. The current study focuses on the relationship between accumulated dry matter and Tt accumulation in the vegetative stage of each of the four grass species.

2.2.2 Describing plant growth and development using thermal time

Accumulated Tt has been used successfully as a predictive tool within plant growth and development models. Hutchinson *et al*. (2000) described long-term (1950-1961) dry matter production (Radcliffe and Cossens, 1974) using a single base temperature (Tb) of 4 °C for a mixed species pasture. Mills *et al*. (2006) calculated a Tb of 3 °C and an optimum temperature of 28 °C and used them to calculated the Tt requirement of + and −N 'Wana' cocksfoot. They showed that, temperature adjusted growth rates of +N cocksfoot pastures, increased (P≤0.001) at an almost near constant rate of 7.2 kg DM °Cd^{-1} ha^{-1}, compared with 3.2 kg DM °Cd^{-1} ha^{-1} for −N pastures, once moisture was non-limited (Figure 2-1). This implied that, while + and −N treatments accumulated the same amount of Tt under the same environment, +N cocksfoot pasture production was 44% higher than −N. The difference between temperature adjusted growth rates for irrigated treatments with and without N fertiliser was counted for as the ratio between actual N percentage and the optimum amount of N (Lemaire *et al*., 1989). Pastures which had no N fertiliser added to the soil, experienced a constant N deficit with about half the N content of fertilised treatments (Mills *et al*., 2006).

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Figure 2-1 Accumulation of dry matter production for irrigated +N (●) and irrigated -N (○) pastures as a function of accumulated thermal time (Tt) with a base temperature of 3 °C for a 'Wana' cocksfoot monoculture at Lincoln University, Canterbury, New Zealand (Mills *et al*., 2006).

In dryland pastures, moisture stress in addition to N deficiency also limits pasture production and quality. In the current study, the combined effects of moisture (using two soil types, which differing in PAWC) and N (±N) on seasonal and annual dry matter production of monocultures of brome, cocksfoot, perennial ryegrass and tall fescue are quantified (Objective 3, Chapter 4). Then, the relationships between accumulated thermal time and accumulated DM production of monocultures under different levels of moisture and N are defined (Objective 4). Understanding the effects of environmental variables on dryland pastures production is complicated by possible interactions among these variables. As a result, once yield reductions occur, quantifying these variables helps to identify the responsible mechanism (Jamieson *et al.*, 1998). This allows a more accurate conclusion compared with an empirical description which may not work under different environmental conditions. In this study, luxurious amount of N fertiliser (Section 4.7.7) applied to +N plots are used to determine the potential yield of these dryland pastures in a similar environment but under different moisture conditions, produced by the use of two sites. This is then compared with the control treatments (−N plots) at each site. The physiological effects of moisture stress and N status are reviewed in Sections 2.3 and 2.5.

2.3 Physiological responses to moisture stress

2.3.1 Leaf area, canopy expansion and radiation interception

Tissue expansion is more responsive to water deficit than stomatal conductance (Hsiao *et al*., 1976; Passioura, 1988; Sadras *et al*., 1993). Therefore, reduced leaf area is the first and most obvious response of plants to restricted water availability (Sadras *et al*., 1991; Sadras *et al*., 1993). Reductions in canopy expansion rate or increased rates of leaf senescence reduce the total gas exchange area (Johns, 1978). In contrast, direct reductions in gas exchange occurs through reduced stomatal conductance which is related to the extent to which vegetation is connected to its surrounding atmosphere (Jarvis and McNaughton, 1986).

Even mild moisture stress conditions and reduced WP (WP) result in a progressive decrease in cell turgor pressure. This reduces cell expansion and causes the formation of smaller leaves (Hsiao, 1973; Hay and Walker, 1989). Less light is intercepted because the canopy is below the critical leaf area index (95% interception) for longer and therefore yield is reduced (Johns, 1978). Water deficit in perennial ryegrass has also inhibited the production of tillers, increased the number of dead tillers and reduced leaf appearance and the rate of leaf extension (Barker *et al*., 1985). For example, when the potential soil moisture deficit was 100 mm, the growth rate of water-stressed ryegrass was 1.4 g m^2 d^{-1} compared with 4.0 g m^2 d^{-1} when irrigated to maintain the potential soil moisture deficit at less than 50 mm. In contrast, irrigated cocksfoot produced 4.0 g m^2 d^{-1} when irrigated and 3.0 g m^2 d^{-1} under the water- limited conditions. The leaf extension rate of both species decreased by 40% under moisture-stressed conditions compared with irrigated plants, but dryland cocksfoot had 19.2 tillers per plant compared with 9.6 tillers per plant for perennial ryegrass. Cocksfoot may have intercepted more PAR than ryegrass under these dryland conditions (Norris, 1982) but this was not measured. To examine that, in this research, the amount of intercepted photosynthetically active radiation (R/RO) and RUE are quantified as indicators of responses to moisture stress. The results for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue are compared to assess their responses to different levels of moisture stress. The level of stress was created by using a shallow Lismore stony silt loam (Ashley Dene) and a deep silt loam (Ladbrooks) soils at separate locations.

2.3.2 Measurement of light interception

Equation 2-1 highlights that DM production is affected by the amount of intercepted PAR (R/RO) and RUE, particularly after canopy closure. However, for pastures, an estimate of the increase in R/RO over time is required for each defoliation event. This is dealt with in Chapter 6 where canopy expansion and light interception by pastures are quantified (Objective 8). Then, Objective 9 was to investigate whether reported yields at the different sites were affected by radiation interception and/or RUE.

Several devices and methods are available to measure canopy radiation interception in the field. However, in pastures, practical application is difficult and comparing results usually is confounded due to differences in methodology (Faurie *et al*., 1996; Castro and Fetcher, 1999; Nouvellon *et al*., 2000; Sinoquet *et al*., 2000). Moreover, conventional devices do not detect differences between dead material and green herbage (Wilhelm *et al*., 2000). Another issue is inaccuracy of measuring the amount of light intercepted by short pasture species, so, measurements are mostly underestimated. The same issue must be addressed with residual biomass. In this study, light interception measurements were taken at the end of each regrowth cycle at Ladbrooks and Ashley Dene.

To estimate the amount of intercepted light by each pastures residual biomass, a separate calibration was made based on residual biomass and plant height as explained in Chapter 6.

2.1 Plant available water content (PAWC)

Soil moisture supply is the result of both soil and root characteristics (Jamieson and Ewert, 1999). Soil texture affects the soil pore size distribution which determines the quantity of water the soil can store. PAWC is defined as the difference between the drained upper limit (DUL) and the lower limit (LL) of moisture extracted by a mature crop which has fully explored all soil moisture to maximum potential rooting depth (McLaren and Cameron, 1990). The characteristics of the two soil types used in the current study were described by Webb (2000). Both Wakanui silt loam (Ladbrooks site) and Lismore stony silt loam (Ashley Dene site) were derived from a similar parent material; soil texture and pore size are comparable. However, the PAWC for the Wakanui silt loam is 150 mm m^{-1} but it is only 50 mm m^{-1} for the Lismore stony silt loam soil. The difference is due to the shallow (<0.18 m) topsoil and higher stone content of the Lismore stony silt loam soil resulting in a reported DUL volumetric moisture content of ~10% in the sub soil layers compared with ~30% for the Wakanui silt loam.

Sim (2014), investigated the effects of different moisture levels on dryland lucerne at two sites (Iversen and Ashley Dene sites) with different PAWC. The PAWC to 2.3 m depth was 130 mm and 360 mm for the Ashley Dene and Iversen sites, respectively (Figure 2-2). Lucerne was able to extract moisture to a depth of at least 2.3 m at both sites. However, total water use by lucerne at the Iversen site was 328 mm compared with 131 mm at Ashley Dene.

At Ashley Dene, mean DUL for the soil profile to 2.3 m depth was 295 mm (calculated on a 1 m basis), compared with 725 mm for Iversen. Distribution of water down the soil profile differed between sites with 35% of total water in the top 0.5 m at Ashley Dene compared with 20% at Iversen site, which had a consistent PAWC of ~360 mm m^{-1} (Sim, 2014).

Figure 2-2 Upper (■) and lower (□) water extraction limits to 2.3 metres for mature lucerne at Ashley Dene (A) and Iversen sites (B) at Lincoln University, Canterbury, New Zealand. Shaded areas and numbers represent plant available water content (Sim, 2014).

In the current study, a similar approach was used to create different levels of moisture stress. Soil water extraction was considered for grass species sown in a low PAWC soil at Ashley Dene and a high PAWC soil at Ladbrooks. In addition to soil type, differences in root characteristics of the plants affects PAWC as explained in Section 2.1.1.

2.1.1 Plant variations in root systems and maximum water extraction depth

In water-limited environments, the availability of moisture and nutrients for plants depends on climatic and edaphic factors and the depth, lateral spread and axil resistances of roots (Casper and Jackson, 1997). Root density and depth determine the volume of soil which can be exploited for moisture. These factors determine variations in PAWC among different soil types and species in the same soil (Jury *et al*., 1991).

Garwood and Sinclair (1979) showed that total root length density of dryland cocksfoot was 13% less than dryland perennial ryegrass in the 0-0.6 m zone. Alao, irrigated cocksfoot had 50% fewer roots than dryland cocksfoot pastures in the top 0.1 m of the profile. Wilman *et al*. (1998) and Garwood and Sinclair (1979) found a larger root system for tall fescue than perennial ryegrass when cutting intervals were five and six weeks. Root length and water extraction depth of rough stalked meadow grass was 40 cm compared with 70 cm for timothy and cocksfoot and 80 cm for perennial ryegrass. Tall fescue extracted water at the deepest depth of 100 cm. Water extraction depth paralleled yields obtained during moisture stress conditions; tall fescue showed greatest tolerance to moisture stress especially under a six-week cutting interval, due to more numerous roots at depth than in timothy, cocksfoot or

perennial ryegrass. Drought "tolerance" in these grasses appeared largely determined by the ability of their roots to exploit soil for moisture.

Garwood *et al*. (1979) reported that in a two year old stand, dryland ryegrass produced 2.3 t ha^{-1} while cocksfoot produced 15% less DM and had 35% less total root biomass on a 0.8 m sandy loam soil. In contrast, Evans (1978), in an 8-10 month-old stand, reported that ryegrass pasture yield was 60% less than cocksfoot (12.1 t ha^{-1}) over the same period of time due to the 15% lower total root length density of perennial ryegrass within 0-1.4 m in a 4.0 m deep sandy loam soil. Both Garwood and Sinclair (1979) and Evans (1978) showed a significant reduction in root length density as soil depth increased. Based on these results, success of cocksfoot in dryland systems could be due to access to soil moisture at depth because of a dense, deep root system, or rapid use of topsoil water due to a dense and shallow root system.

To investigate the mechanisms of soil water extraction (depth and root density) for dryland brome, cocksfoot, perennial ryegrass and tall fescue the current study compared water extraction profiles at two sites with different PAWC. This approach was used because direct measurement of plants root is difficult and inaccurate. Objective 5 in Chapter 5 is to quantify PAWC of brome, cocksfoot, perennial ryegrass and tall fescue pastures at these contrasting sites.

2.2 Quantifying the effects of moisture on yield

The association between roots and crop yield in moisture-stress conditions can be analysed using Equation 2-3 (Passioura, 1983). In this equation, yield is a function of crop water used (WU, mm) multiplied by water use efficiency (WUE, kg DM mm^{-1}) (Tanner and Sinclair, 1983). The accuracy of this method depends on whether WU is based on transpiration, actual evapotranspiration (AET) or potential evapotranspiration (PET).

Equation 2-3 $\mathrm{DM\ yield} = \mathrm{WU} * \mathrm{WUE}$

Under conditions wherein precipitation from rainfall (R) and/or irrigation (I) is insufficient to replace water depleted from the profile, soil moisture deficits increase (Penman, 1970). The variation in soil moisture content is a result of the crop water and energy balance. The water balance (Equation 2-4) shows that the change in soil water content (ΔSWC) depends on the balance between evapotranspiration (ET) losses and precipitation from rainfall and irrigation (PR+I).

Equation 2-4 $\Delta\mathrm{SWC} = \mathrm{PR} + \mathrm{I} - \mathrm{ET}$

The amount of rainfall and irrigation are simply measured. However, ET values depend on a complex relationship among weather, plant surface and soil factors (French and Legg, 1979). Equation 2-5 is

used to quantify these factors. In this equation, PET is potential evapotranspiration (mm), Δ is the slope of saturation water vapour pressure against temperature (mb K^{-1}) at a known air temperature, γ is the psychometric constant (mb K^{-1}), H is the net radiation (J/m^2/d), p is the density of water (kg/m^3) and λ is the latent heat of vaporisation (J kg^{-1}) at a given air temperature (French and Legg, 1979).

Equation 2-5 $PET = \frac{\frac{1000\Delta H}{p\lambda + \gamma + Ea}}{\Delta + \gamma}$

Under normal conditions, in which water is a non-limiting factor for growth, soil moisture deficits calculated by this method are reasonably accurate (French and Legg, 1979). However, when plants are prone to moisture stress, most of the assumptions used to calculate the extent of the soil moisture deficit do not apply. This results in an overestimation of actual evapotranspiration (AET) in water-stressed crops (Tanner and Sinclair, 1983) because PET is purely a measure of atmospherically-driven demand (French and Legg, 1979; Hopmans and Bristow, 2002).

For several pasture species, Garwood and Sinclair (1979) reported that, before harvest PET was 2.9 mm d^{-1}; this overestimated AET by 21%. After harvest, PET was 3.5 mm d^{-1}compared with AET of 0.8 mm d^{-1}. Because PET does not account for differences in the total available water holding capacity of the soil (TAWC) or plant demand, soil moisture deficits calculated using PET will continue to increase until rain or irrigation is applied. In contrast, AET will increase and then plateau when pasture growth slows/ceases because there is i) low demand and/or ii) insufficient plant available water for growth to continue (French and Legg, 1979; Hopmans and Bristow, 2002).

2.2.1 Water use efficiency (WUE)

The concept of water use efficiency (WUE) is usually regarded as a main determinant of crop production under stress and even as a part of crop resistance to drought stress. It has been used to show that dryland plant production can be raised per unit water used, following a strategy of ‘‘more crop per drop’’ (Blum, 2009). In most of the studies WUE is based on above ground yield and partitioning to the roots is not considered. Variation in root: shoot ratio or seasonal modifications in partitioning which may happen in perennial pasture species, are not accounted for in these calculations (Teixeira, 2006). Since root material may be lost when samples are washed, it is difficult to accurately measure root dry matter of pasture species with fibrous root systems. In this study, the slope of the regression between above ground DM yield and the quantity of water (mm) used (Section 2.3) to produce that yield is regarded as WUE. Plants which are exposed to a moderate water deficit show an increase in WUE (Silcock and Wilson, 1981). This increasing efficiency is due to a reduction in the quantity of water associated with evaporative loss and can change among species and in different environments. Consequently, instead of the calculations based on evapotranspiration, transpiration

efficiency (Tanner and Sinclair, 1983) can be used to define WUE. Increasing yield is due to using more water for transpiration instead of the soil or leaf evaporation components of evapotranspiration. Under drought stress, variations in WUE may reflect the inaccuracy of PET when PET is used to quantify evapotranspiration. In the current study, WUE will be calculated using Equation 2-6, where ET is transpiration (mm) which is actual water use measured in the field (Section 5.2).

Equation 2-6 $\mathrm{WUE} = DM/ET$

2.3 Measurement of soil water content

To measure water content, gravimetric sampling is the most accurate method. However, this requires that soil samples be removed from a bulk soil. In *situ* methods which measure soil water content include radioactive methods such as the neutron probe (Gardner and Kirkham, 1952) and gamma ray attenuation (Reginato and Van Bavel, 1964). These methods are reasonably accurate and non-destructive but they need calibration for each soil type and safety precautions. Davis and Chudobiak (1975) developed an alternative, non-destructive technique to quantity soil water content using time domain reflectometry (TDR). TDR defines the dielectric constant of an object by inserting electrodes. Topp *et al*. (1980) suggested an experimental association between dielectric constant and the amount of volumetric water content in soils with varied textures.

In this research SWC in the top layer (0-0.2 m) were measured with a time domain reflectometer (TDR) and neutron probe access tubes were installed to quantify soil water content to a depth of 2.3 m at Ladbrooks and 2 m at Ashley Dene in each plot (Section 5.2). This can be used to assess differences among species in their ability to extract moisture as well as measure maximum extraction depth.

2.4 Quantifying moisture stress

Plant moisture status can be quantified by measuring the WP of the leaves (Ψ; MPa), a measure of cell turgor pressure (Gonzalez-Dugo *et al*., 2010). When the plant is not able to extract soil moisture to meet its water needs, water demand is reduced. However, this method is time consuming (Gonzalez-Dugo *et al*., 2010) and varies with environmental conditions.

Another method of quantifying moisture stress is defining the degree of stress as an index of transpiration relative to transpiration demand (E_T/EP_T) (Jamieson *et al.*, 1998a; Robertson *et al.*, 2002). EP_T is daily EP multiplied by crop cover (French and Legg, 1979). A value of 1.0 demonstrates $E_T = EP_T$, and there is no moisture stress. This is important because the critical limiting deficit method is site and species specific. Sim (2014) quantified the occurrence and time of moisture stress for regrowth of a lucerne crop by plotting the relationship between E_T/EP_T and soil moisture deficit at two sites. At Iversen on a deep Wakanui soil, the limiting deficit was 243 ±42 mm beyond this level the ET/EPT was

reduced by 0.005 per mm of soil moisture deficit. However, at Ashley Dene with a Lismore stony soil, ET/EPT did not reach 1.0. The maximum crop water demand supplied by the soil was 71% when the soil moisture deficit was <81. Therefore, he concluded that the concept of critical deficit was not applicable on a Lismore stony soil type. In the current study, the relationship between actual soil moisture deficit and ET/EPT is examined to investigate if the method can be used to define the critical limiting deficit for dryland brome, cocksfoot, perennial ryegrass and tall fescue pastures on each soil type.

2.5 The combined effects of moisture and nitrogen on pasture grass species dry matter production

Section 2.2.2 showed that, under non-limiting N and adequate soil moisture, cocksfoot had a potential yield of 21.9 t DM ha^{-1} yr^{-1} at this location (Mills *et al*., 2006; Peri *et al*., 2002).

Figure 2-3 shows the effect of temperature and moisture on the production of dryland cocksfoot pasture grown in a Templeton silt loam soil (*Udic Ustochrept*, USDA Soil Taxonomy), with (+N) and without N application (Mills *et al*., 2006). The results showed that, in dryland conditions, the production of −N cocksfoot pastures was always lower than +N pastures (Figure 2-3). However, severe drought (when critical soil moisture deficit was <78 mm) caused a consistant decrease in yield of the dryland + and −N pastures at a rate of 1.45% DM mm^{-1} relative to their respective irrigated control pastures. In 2004/05, this happened from 8/11/2004 to 1/12/2004, for a total of 230 °Cd (320-550 °Cd) in both dryland + and −N pastures (Figure 2-3). This shows that, under severe drought conditions, even when N is not a limitation to growth, production will only proceed at the rate allowed by the most limiting factor, in that case moisture (Mills *et al*., 2006).

The current study is similar to Mills *et al*. (2006) which investigated the potential yield for +N irrigated and dryland 'Wana' cocksfoot and compared it with the control (−N) under the same conditions. However, this research accounts for the potential yield for dryland perennial pasture production for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue under two soil types which are different in PAWC. Pastures at Ashley Dene are expected to be mostly under moisture stress due to the Lismore stony silt loam soil and its lower PAWC compared with Ladbrooks with a Wakanui soil type.

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Figure 2-3 Accumulated dry matter production (kg DM ha^{-1}) for 'Wana' cocksfoot in dryland plus N pastures in (a) 2003/04 (▼) and (b) 2004/05 (▽) and dryland minus N pastures in (c) 2003/4 (●) and (d) 2004/5 (○). Thermal time was accumulated (Tb= 3 °C) and dashed vertical lines show the period of time in which the critical limiting deficit (78 mm) was exceeded (Mills *et al*., 2006).

When tall fescue was compared with seven grasses within the *Lolium Festuca* L. complex, it was shown to be the most drought-tolerant grass (Ebrahimiyan *et al*., 2013). Turner *et al.* (2012) showed that tall fescue maintained productivity and displayed characteristics that contribute to persistence under drought conditions. The greater leaf DM by tall fescue in their study was because of its greater water absorbance (69 mm in the second regrowth period, versus 53 and 48 mm for cocksfoot and perennial ryegrass, respectively) and reflects its more efficient 'dehydration delay' approach in response to moisture deficit, as defined by Volaire *et al*. (2009). However, no mechanism was given for 'dehydration delay' for tall fescue. Their results suggested that leaf DM production by the three species was consistently in the order: tall fescue > perennial ryegrass > cocksfoot. Neal *et al*. (2009) also showed the same results under optimal irrigation in subtropical field conditions. Leaf DM was lower for cocksfoot compared with perennial ryegrass and tall fescue. However, cocksfoot was not as sensitive as tall fescue and perennial ryegrass to soil moisture deficit as it had the least relative reduction in DM due to moisture stress. This means that, while perennial ryegrass showed a 78% decrease in leaf DM (the 33% water treatment compared with the fully watered treatment), leaf DM reduction in cocksfoot was 52%. During the recovery stage, there was a 58% difference in leaf DM between perennial ryegrass which was watered at 33%, compared with the fully watered treatment. There was a 35% difference for cocksfoot and tall fescue which showed an intermediate response to soil moisture deficit. Tall fescue and perennial ryegrass displayed a trend of increasing leaf DM with

increasing water distribution before the recovery phase. In both periods of treatment and recovery, perennial ryegrass did reasonably well regarding absolute leaf DM. There were no visible signs of plant wilting or any reduction in leaf elongation (in any of the species) under the 33% water treatment. Nevertheless, variation in stubble DM and the number of tillers suggest that moisture stress decreased emerging tillers, mainly in perennial ryegrass. This increased the sensitivity of perennial ryegrass to reduced soil moisture (i.e. presenting the highest relative decrease in DM under water deficit conditions) and tall fescue. Based on their results, the DM production of perennial ryegrass would likely decreased more with severe moisture deficit treatments or if a deficit-irrigation approach been used, increasing the period between watering events (Turner *et al*., 2012). Black and Murdoch (2013) reported 25–29 kg ha^{-1} mm^{-1} of water for perennial ryegrass in dryland and irrigated ryegrass/white clover pastures, when 400 kg ha^{-1} yr^{-1} of N fertiliser was added. As expected, N fertiliser increased dry matter production across irrigated and non-irrigated treatments. In contrast, when N fertiliser was not applied, WUE was 61–69% of the maximum (18-20 kg DM ha^{-1} mm^{-1}). Near infrared reflectance spectroscopy (NIR) tissue analysis indicated a higher N content of ryegrass in + N treatments, than in the control. Nitrogen deficiency decreases the metabolic N pool rather than structural N. Reductions in the formation of chlorophyll and Rubisco cause a decrease in gross photosynthesis and radiation use efficiency. In the current study, the combined effects of moisture and N in relation to accumulated Tt on production of brome, cocksfoot, perennial ryegrass and tall fescue are described (Objective 3, Section 1.4).

2.6 Plant nutritional value

The nutritive value of a pasture grass species for ruminant production is a function of voluntary intake and feed utilisation (Stone, 1994). These two parameters are not independent (Black, 1990). The amount of energy which is available in plant tissue, protein levels within the tissue and plant digestibility are the most important factors that affect nutrient value (Stone, 1994). Pasture grazing can complicate analysis due to uneven pasture utilization and return of nutrients in urine and dung. In this study the stony silt loam soil at Ashley Dene was grazed because mechanical removal was impractical. However, the Ladbrooks site was mechanically harvested for each rotation.

2.6.1 Nitrogen metabolism

Nitrogen (N) is the most important nutrient for plant growth (Grindlay, 1997; Azam, 2002). In the photosynthetic system, N plays a key role in chlorophyll, Rubisco and protein synthesis. Moreover, it can promote cell division and tillering and is an essential component of amino acids which are precursors to protein formation and necessary for the formation of enzymes and co-enzymes (Hay and

Walker, 1989; McLaren and Cameron, 1990). The net effect of N supply can affect both leaf area and photosynthesis (Novoa and Loomis, 1981).

2.6.2 Dietary metabolisable energy

Metabolisable energy is often used as an indicator of the available energy of pasture. Forage ME values can be used to indicate animal production from defined intakes or to estimate dry matter intake (Waghorn, 2007). Although ME values can be used to estimate "quality" within a feed type (e.g. over a growing season), they are not a measure of nutrient content (Waghorn, 2007).

Determination of ME is by combustion in a bomb calorimeter. However, near infra-red spectroscopy is now commonly used as an indirect estimate. Pasture ME changes with season, species and botanical composition. Water deficit has also been shown by Mills (2006) to reduce ME (Table 2-1). In 'Wana' cocksfoot, the interaction of N*Y had an almost similar effect on the annual ME yield of the −N treatments in 2003/04 (72 GJ ME ha^{-1}) and 2004/05 (66 GJ ME ha^{-1}) but a 7% rise for +N treatments between 2003/04 (172 GJ ME ha^{-1}) and 2004/05 (183 GJ ME ha^{-1}) (Table 2-1). In addition to the interaction of N*Y, N fertiliser was the major factor that determined differences and characterised 83% of the treatment sum of squares compared with <1% for the interaction; ME of cocksfoot ranged from 10.2 to 12.4 MJ/kg DM. In almost all cases, treatments showed no differences in winter, but N increased ME content in the summer. In the second year (2004/05), there was a minor deviation than in the first year, but ME tended to be greater over winter and, in dryland +N pastures, in the autumn. This is because lower temperature in autumn and early spring restricts movement of simple sugars that then accumulate in the vacuole, to developing organs (Nicol, 1987).

Table 2-1 'Wana' cocksfoot monoculture annual metabolisable energy (ME) and yield (GJ ha^{-1}) grown at Lincoln University, Canterbury, New Zealand in 2003/04 and 2004/05 (Mills *et al*., 2006).

Treatment	2003/04	2004/05
I+N	202	204
I−N	91	86
D+N	143	161
D−N	53	45
Effect	N*Y	
Significance	**	
LSD (P≤0.05)	10.0	

Note: Treatments are irrigated (I) or dryland (D) as the main plots and +N and −N as sub-plots. Levels of significance are: 0.05 (*), 0.01 (**), and 0.001 (***).

In Canterbury, New Zealand, cocksfoot pasture quality parameters were significantly affected by N. Low N led to an increased quantity of senesced and/or diseased leaf tissue which in turn reduced dry matter production and nutritive value and resulted in reduced pasture utilization (Edwards *et al*., 1993).

2.6.3 Crude protein (CP) and fibre digestibility

Percent crude protein (% CP) is calculated as the %N multiplied by 6.25; plant protein is approximately 16% N. Since insufficient protein will reduce animal and plant production, N is the most important factor irrespective of ME availability. For animals, CP in excess of nutrient requirements is converted to urea for excretion (Waghorn, 2007). Neutral detergent fibre (NDF) and CP content affect pasture digestibility and are the main determinants of ME in forage diets. NDF% of temperate forages range from 25% in clover and 35% in immature grass, 43-53% in typical pastures to 65% or more in mature grass (Waghorn, 2007). The values for CP range from 10% in senescent grass to 30% in fertilised pasture and legumes.

In this research, the effects of N on dryland pasture quality (ME and CP) under different levels of moisture were measured using near infera-red spectroscopy as described in Section 4.8.4.

2.7 Major New Zealand pasture species

There are many improved grass, legume and forage cultivars available for New Zealand farmers. More than 60 different perennial/long rotation ryegrass cultivars with different endophyte choices (nil, wild type or modified 'novel'; see above) are available. Flowering time, cool season activity and ploidy levels differ among these cultivars. There are at least 15 cultivars of tall fescue, cocksfoot, bromes (*Bromus* spp.) and timothy. The dominant sown pasture legume is white clover with more than 20 cultivars that differ in terms of leaf size and the stolon density (Moot *et al*., 2009). Several cultivars of red and subterranean clovers and more than 10 lucerne cultivars are commercially available or registered for use. Chicory (*Cichorium intybus* L.) and plantain (*Plantago lanceolata* L.), are commonly used pasture forbs (Caradus, 2008).

2.8 Species selection

Environmental conditions have a key role in choosing appropriate pasture species. Perennial ryegrass is the most suitable pasture grass in moist, lowland environments. However, it is less suitable for dryland South Island High Country regions. Perennial ryegrass, cocksfoot, grazing brome (*Bromus stamineus* L.) and pasture brome (B. *valdivianus* L.) are grass recommended in moist hill country with lotus, white and red clovers are the recommended legumes, depending on soil pH. The east coast areas of New Zealand represent about 3.0 M hectares which are subjected to regular extended periods of

summer/autumn water limitation, and this is expected to increase due to the climate change. The dominant sown legume in these pastures is still white clover even though it is not well adapted to soil moisture deficit in summer (Knowles *et al*., 2003). If water limitation becomes more persistent, and more regular, white clover production and persistence will be even less reliable. Leading farmers will show greater reliance on annual pasture species such as subterranean clover and deep rooted perennials such as lucerne (Moot *et al*., 2009). The dryland pasture group at Lincoln University has undertaken significant research on these plants but less work has been done on drought tolerant perennial grasses (Moot, 2012) which is the focus of this research.

2.8.1 Cocksfoot

Cocksfoot is a productive grass which is able to tolerate drought conditions and grows strongly during warm seasons (Norris 1982). Nevertheless, if it is not frequently grazed, it can become unacceptable and coarse to stock. In comparison with perennial ryegrass, cocksfoot is slower to establish (Charlton and Stewart, 1999).

In areas with water limitation, moderate fertility light and free-draining soils cocksfoot is one of the most suitable grasses. Cocksfoot can be regarded as an endophyte-free grass and can be safely grazed during summer. However, the quality of its forage is considered lower than that of perennial ryegrass and it can be particularly poor in the presence of seedheads. Therefore a goal of grazing management should be to prevent development of excessive seedheads (Moloney, 1993).

2.8.2 Tall fescue

Tall fescue is a perennial and deep-rooted grass (Stephenson and Posler, 1988) which has high productivity (Charlton and Stewart, 1999), water deficit tolerance (Charlton and Stewart, 2006), heat tolerance and summer growth (Reed, 1996), with tolerance of wet soils (Reed, 1996) and a responsiveness and positive reaction to irrigation (Lowe and Bowdler, 1995).

When tall fescue was compared with seven grasses within the Lolium–Festuca complex, it was shown to be the most drought-tolerant (Ebrahimiyan *et al*., 2013). Under drought conditions, it maintained productivity and displayed characteristics that contributed to persistence (Turner *et al*., 2012). These include consistently higher leaf dry matter than perennial ryegrass and cocksfoot, which indicates smaller decreases in growth under water limitation and an ability to recover faster upon re-watering.

Compared with cool-season turf grasses, tall fescue is also regarded as more drought-tolerant (Shaffer *et al*., 1994). Tall fescue was able to survive under long periods of dry summers and it could be as a result of its potential for summer dormancy (Norton *et al*., 2006). Also, tall fescue may be naturally

infected with *Neotyphodium* endophytes, which are able to improve its performance under different stress conditions such as drought stress (Mirlohi *et al*., 2004).

In pastures sown with more than one species, tall fescue is more likely to be legume friendly to clovers in comparison with ryegrass. This is because of the greater required thermal time for field emergence and less vigorous seedling growth of tall fescue in comparison with perennial ryegrass (Moot *et al*., 2000).

Compared with perennial ryegrass and some other grasses, tall fescue is slow to establish and more sensitive to drought conditions in the establishment period (Gibson and Newman, 2001). Variance in seedling vigour of tall fescue and ryegrass is ascribed to differences in their ability to mobilise and consume endosperm supply (Brock *et al*., 1982). In this research, to compare the rate of germination in the establishment phase under different moisture levels and temperatures among the four pasture grasses a "Hydrothermal time" experiment was conducted (Chapter 3).

2.8.3 Brome grasses

Compared with perennial ryegrass, different species of brome provide more production in cool seasons. However, this plant is more vulnerable to insect pests and sensitive to soils with high acidity or poorly drained regions (Moot *et al*., 2000).

2.8.4 Prairie grass

Prairie grass is a short-lived perennial which has large leaves and tillers and is originally from South America. It provides higher winter/early spring growth than perennial ryegrass and is drought tolerant (Charlton and Stewart, 1999). This grass shows a decrease in maximum rates of photosynthesis under drought conditions which may continue for more than seven days or even to the end of a drought period (Heckathorn and DeLucia, 1994; Heckathorn, 1995). It is regularly used as a special-purpose pasture for high quality winter and summer feed. In contrast with most other grasses, its large-seeded panicle flowerhead is palatable to grazing stock. Prairie grass offers productive high quality pasture for 2–4 years.

2.8.5 Pasture brome

Compared with prairie grass, pasture brome is more persistent under grazing. It's a moderate winter growth species but provides strong spring–summer growth with drought tolerance. Similar to other brome species, it does not tolerate pugging or waterlogging.

During summer, it has many average sized tillers and is able to produce a confined number of aftermath inflorescences (Stewart, 1992). This plant does not have dormancy in summer. However,

since it has numerus vegetative tillers in summer, and also due to the natural ability of the plant to persist under high temperatures, it is able to persist in summer (Laude, 1953). It has a moderately deep root system and superior performance to perennial ryegrass under moisture stress (Stewart, 1996). Pasture brome is appropriate to dry east coast and inland areas of New Zealand, where it is useful as a special-purpose pasture for quality summer feed (Charlton and Stewart, 1999).

2.8.6 Perennial ryegrass

Perennial ryegrass is the most common temperate grass in New Zealand and is able to grow in a wide variety of soils with moderate to high levels of fertility. It is usually sown with white clover and several other pasture species and is easy to establish and manage. It requires moist, fertile conditions and is able to tolerate treading and hard grazing. In comparison with deeper-rooted grasses which may maintain high levels of dry matter production during hot dry summers, the performance of perennial ryegrass is low. In areas with high humidity in summer, it cannot withstand crown (*Puccinia corona*) or stem (P. *graminis*) rust diseases which lead to decreased production, feed quality and persistence.

Perennial ryegrass contains an endophytic fungus which produces three main toxins within the plant, primarily near the shoot base and in seedheads:

• Peramine: is able to prevent feeding by Argentine stem weevil (*Listronotus bonariensis*), and some other pests; significantly increases perennial ryegrass persistence throughout much of New Zealand. It is less important in cool, moist regions such as Southland, the West Coast and inland South Island regions.

• Lolitrem B: causes ryegrass staggers in grazing animals. The levels of Lolitrem B vary during the year, but mostly reach harmful levels in late December in dryland areas, and can remain so until late autumn.

• Ingesting Ergovaline: by animals causes heat stress and blood circulation difficulties. Levels also vary significantly over the year and reach the maximum level during summer. Ergovaline can affect livestock when levels are as low as 0.5 ppm, though they peak at almost 2 ppm.

Perennial ryegrass is regularly used in New Zealand livestock farming systems. Low-endophyte types are prefered for deer and horses since they are more sensitive to ryegrass staggers. There are now a range of alkaloids available within cultivars of perennial ryegrass. These include ‘NEA2’, ‘AR1’ and ‘AR37’ which are all claimed to provide insect resistance with lowered animal health risks.

Based on the literature, tall fescue, cocksfoot and brome have all demonstrated greatest drought-tolerant and higher production compared with perennial ryegrass, especially when N is not limiting. After a drought period, these grasses are also able to recover faster than perennial ryegrass/ white

clover pastures which are usual in New Zealand. Thus, in the current research, 'FinesseQ' for tall fescue, 'SFR36-009' an unnamed breeding line for cocksfoot, 'Stellar AR1', perennial ryegrass and 'Bareno 9045D' brome were used and their production was compared both with (+N) and without N (−N) under different moisture levels. Since it is impossible to segregate and recognize the effects of endophyte on plants drought resistance, nil endophyte was used in the current study.

To compare species it is important to know the physiological basis of decreasing dry matter production due to moisture stress and therefore to define the mechanisms that cause yield reductions. Chapters 5 and Chapter 6 account for the mechanisms affecting dryland pastures production.

2.9 Factors that influence pasture establishment

The ability of a seed to germinate and become a self-supporting plant is a most important component of pasture establishment. Under field conditions, soil moisture and temperature regulate germination rate and percentage (Baskin and Baskin, 1998). In this section, previous studies are reviewed regarding a mathematical model to describe the effects of temperature and/or moisture on seed germination.

2.9.1 Temperature effect on seed germination

The importance of temperature and its effect on plant development have been described and quantified as "thermal time" (Tt) (Arnold and Monteith, 1974). Tt is defined as the number of thermal units or heat units or growing degree days (GDD), accumulated over a given period of time. Thermal time requirements for germination remove location-specific calendar day-based periods, regardless of the ecological niche. Thermal time accumulation involves the definition of "cardinal" temperatures; base (Tb), optimum (To) and ceiling (Tc) temperatures for phenological development stages of a particular species (Angus *et al*., 1981).

For germination, the optimum temperature (To) is the temperature (or range of temperatures) at which the highest germination percentage is achieved by a seed population in the shortest possible period of time (Orozco-Segovia *et al*., 1996). For tall fescue and perennial ryegrass Zhang *et al*. (2013) reported that, maximum germination rate occurred when temperature was between 12 to 32 or 35 °C for perennial ryegrass. The To range of tall fescue cultivars was narrower, 18 to 26°C for 'Flecha AR542' and 15 to 29 °C for 'Advance'.

Temperatures which are higher than the optimum (T>To) are defined as "supra-optimal". The optimum temperature for seed germination (To) of many temperate species is ≤20 °C (Bradford, 2002). As a result, even if temperature is suitable for seedling growth, an increase above the optimum leads to a rapid decline in the proportional rate of seed germination (Bloomberg *et al*., 2009). Physiological changes occur in seeds at supra-optimal temperatures and these affect the rate of germination and

the proportion of the seed population that will complete germination (Hills and Van Staden, 2003; Argyris *et al*., 2008).

Thermal time requirements of several temperate pasture species have been defined (Moot *et al*., 2000; Lonati *et al*., 2009). Lonati *et al*. (2009) defined the pattern of germination, emergence and leaf appearance of temperate pasture species of six cultivars of clover (*Trifolium arvensis*, *T*. *dubium*, *T*. *glomeratum*, *T*. *striatum*, *T*. *subterraneum*, *T*. *repens*) and four grasses (*Bromus erectus*, *Festuca rubra*, *F*. *ovina*, *Achnatherum calamagrostis*), using thermal time (Tt, °C days). Figure 2-4 shows the effects of temperature on seed germination. Temperature affected ($P<0.01$) both germination rate and final germination percentage. The germination percentage of *Festuca rubra* and *F*. *ovina* was ≥70% at 25.5 °C. However, it decreased to 4% and 24%, respectively at 35.6 °C (Moot *et al*., 2000). For *B*. *erectus* it was similar at 25 °C, but the germination was maintained at 48% at 35.6 °C. Maximum germination for *A*. *calamagrostis* fell quickly from more than 50% up to 16.6 °C to 8.1% at 25.5 °C after which there was no more germination. For all species, except for B. *erectus*, base temperatures (Tb) were below 2.5 °C; Tb for *B*. *erectus* was 5.8 °C. Compared with clovers, all grasses had slower germination rates and a broader tolerance of high temperatures (Lonati *et al*., 2009).

Figure 2-4 A) *Festuca rubra* L., B) *F. ovina* L., C) *Bromus erectus* L., D) *Achnatherum calamagrostis* L. seeds cumulative germination at 10.3 (●), 14.9 (o), 16.6 (▼), 19.2 (▽), 21.9 (■), 25.5 (□), 29.0 (◆), and 35.6 (◊) °C; only (D) *Achnatherum calamagrostis*, (▲) prechilled at 0.5 °C for 13.2 days at and germination at 14.1 °C. Bars show the highest standard error for the final germination percentage (Lonati *et al*., 2009).

2.9.2 Applying mathematical model to describe thermal time

At sub-optimal temperatures, germination can be described based on thermal time units accumulated by the seeds. In this temperature range, germination response to temperature is explained by mathematical models (Feddes, 1972; Covell *et al.*, 1986; Dahal and Bradford, 1990).

Equation 2-7 $\theta T1\ (g) = (T - Tb)\ t\ (g)$

As shown in Equation 2-7, at sub-optimal temperatures, the thermal time required for a given percentile of germinated seeds (θT1 (g)) is equal to the accumulated thermal units in excess of Tb. To linearize the thermal model of Equation 2-7, germination rate (GR) is defined as the inverse of the time taken by the seed to germinate. This can be calculated for any given percentile (g^{th}) of the seeds (1/GR (g) = t (g)) as:

Equation 2-8 $GR\ (g) = 1/t\ (g) = (T - Tb)/\theta T1\ (g)$

The germination rate of different seed percentiles increases linearly when temperatures are above the base temperature (Tb) (Labouriau and Osborn, 1984; Ellis *et al*., 1986). In contrast, increasing

temperature causes a linear reduction in germination rate in the supra-optimal range of temperatures (Angus *et al*., 1981). To describe germination rate in the supra-optimal temperatures (from To to Tc), a similar mathematical model can be used.

Equation 2-9 $\theta T2 = (Tc\ (g) - T)t\ (g)$

or

Equation 2-10 $GR\ (g) = 1/t\ (g) = (Tc\ (g) - T)/\theta T2\ (g)$

θT2 is a thermal time constant at supra-optimal temperatures and Tc values change amongst the seed fractions (g), in a seed population.

GR can be described by linear regression at sub- and supra-optimal temperatures. The slopes of the two regression lines are θT1 (g) and θT2 (g) with the intersections of the two lines with the X-axis (temperature axis) defined as Tb at sub-optimal and Tc at supra-optimal temperatures (Garcia-Huidobro *et al*., 1982). Based on Equation 2-10, at supra-optimal temperatures, different germination rates for different germination percentiles are due to variation among seeds in their ceiling temperatures, while the cumulative amount of thermal units by time remains constant (Gummerson, 1986; Bradford, 1995).

2.9.3 The effects of moisture on seed germination

Most previous studies have aimed to quantify the effect of temperature on seed germination of pasture species. However, in field situations and particularly under dryland conditions, moisture as well as temperature affect seed germination and pasture establishment.

To understand the impact of different moisture levels (WP) on seed germination, the base WP (Ψb) needs to be defined in a similar manner to the base temperature.

The base WP (Ψb (g))

The difference between the seed WP (Ψ) and a physiological base or threshold WP for radicle emergence defines the time to germination of a seed. The base WP (Ψb) is the most negative (lowest) Ψ at which a given seed percentile can germinate (Finch-Savage, 2004). The relationship between seed germination rate and WP has been described as:

Equation 2-11 $\theta H = (\Psi - \Psi b\ (g))\ t\ (g)$

In this Equation, θH is a hydrotime constant, Ψb (g) is the base or threshold Ψ that prevents germination of the gth fraction of the seed population (Gummerson, 1986).

The base WP (Ψb (g)) varies within a seed population. These variations usually can be described by a normal distribution (Bradford, 2002; Finch-Savage, 2004). A normally distributed Ψb (g) means that the median base WP (Ψb (50)) will be equal to the mean Ψb (g) for the seed population. In the current study, the concept of the base WP or Ψb is defined as the base WP for the germination of the 50th percentile of the seed population.

Most studies suggest that Tb is constant or fluctuates little within seed populations (Garcia- Huidobro *et al*., 1982; Kebreab and Murdoch, 1999; Watt *et al*., 2011). Therefore, it can be concluded that, in a seed population, the distribution of seed germination times is due to the variation in Ψb (g) (Bradford, 2002; Finch-Savage, 2004). This means that in a seed population, different thermal time requirements for different seed germination percentiles are caused by differences in the base WPs among the seeds, which follows a normal distribution (Alvarado and Bradford, 2002).

2.10 Hydrothermal time model

By combining Equation 2-7 and Equation 2-11, Gummerson (1986) proposed hydrothermal time as a method to model seed germination. Bradford (1995, 2002) expanded the approach as:

Equation 2-12 $\theta HT = [\Psi - \Psi b\,(g)]\,(T - Tb)\,t\,(g)$

θHT is a hydrothermal time constant (Gummerson 1986; Bradford 1995), that has a unique value for the seed population (MPa°Cd), Ψb (g) is the base WP for the gth percentile in a seed population (MPa), Tb is the base temperature for seed germination (°C) and t (g) is the time for the gth percentile to germinate.

To linearize the hydrothermal model (Equation 2-13), germination rate (GR), was replaced by time to germination (1/GR = t (g) in Equation 2-13:

Equation 2-13 $GR\,(g) = [\Psi - \Psi b\,(g)]\,(T - Tb)\,t\,(g)/\theta HT$

Based on the Hydrothermal time model, under a constant temperature and WP, the gth seed in the seed population will germinate when it has accumulated a certain quantity of hydro and thermal time. Across the sub-optimal range of temperatures, Equation 2-12 and Equation 2-13 show an accurate fitting for all moisture regimes (e.g. Dahal and Bradford, 1994). However, germination rate cannot be predicted accurately for the supra-optimal range predicted by the Gummerson (1986) model in Equation 2-13.

2.10.1 Hydrothermal time models at supra-optimal temperatures

Bradford and Somasco (1994) reported a reduction in seed germination rate of lettuce (*Lactuca sativa* L.) seeds by increasing temperature in the supra-optimal range of temperatures. Also, a limitation in the temperature range for germination of *Orobanche aegyptiaca* L. seeds was observed in a study by Kebreab and Murdoch (1999, 2000) at temperatures above To. Different reasons for inhibition of germination at supra-optimal temperatures have been reported for many species that normally germinate in the autumn or winter (Baskin and Baskin, 1998).

Bradford and Somasco (1994) proposed that a positive shift in base WP at supra-optimal temperatures, has the same effect as reduced Ψ at sub-optimal temperatures. Thus, the germination rate decreases as temperature increases above To. Increasing temperature in the supra-optimal range leads to an upward shift above 0 MPa in Ψb (g) for a fraction of seeds. This means the threshold for that fraction of the population is higher than 0MPa, making them unable to germinate in water (Bradford, 1996). A progressive reduction in germination rate distribution in a seed population could be due to this mechanism.

2.10.2 Decreasing germination rate at supra-optimal temperatures

Section 2.9.1 explained how an increased temperature in the supra-optimal range causes a reduction in the rate and final germination percentages. Thermal denaturation of proteins and consequences on membranes have been suggested as candidate mechanisms for thermo-inhibition, but a small rise above To is less likely to cause this effect. Hilhorst (1998) reported that lipid transition temperatures or molecular mobility of membrane components possibly cause changes in Tc that can arise during release or imposition of dormancy.

Both 'Alvarado and Bradford' and 'Rowse and Finch-Savage' hypothesised that a shift upward towards zero in the base WP values causes a decline in germination rate at the supra-optimal range of temperatures. The standard deviation of the Ψb values about the mean were similar when T>To (Bradford, 2002).

2.10.3 Effects of moisture on pasture species germination

McWilliam *et al*. (1970) reported the effect of six different WPs (-1.6 to 0 MPa) on the final germination percentage and germination rate of seven pasture species when temperature was 23 °C. Perennial ryegrass showed the highest and cocksfoot the lowest germination rate among the other grasses. Perennial ryegrass was the least affected by the reduced WPs. At 23 °C, the final germination percentage of perennial ryegrass remained unchanged by decreasing WP from 0 to -0.8 MPa. At 6 °C, the final germination percentage of tall fescue cultivar 'Flecha AR542' was only 8% and that of

'Advance' was 2%. Perennial ryegrass cultivars showed almost 20% higher germination under the same conditions.

Laboratory experiments including thermal time (TT) and hydrothermal time (HTT) can assess seed germination responses to different temperature and moisture/temperature conditions. In this research, the combined effects of WP and temperature on the germination of brome, cocksfoot, tall fescue and perennial ryegrass are described (Chapter 3, Objective 1).

2.10.4 Relationship between soil water content and water potential

Water status is generally expressed in terms of WP in hydrothermal research. However, it would be more practical to estimate soil moisture content to be able to generalize hydrothermal time results to field conditions.

The relationship of soil water content and soil WP is required for many plant and soil studies. However, measurement of these relationships is difficult, costly and is not usually practical. For many purposes, general estimates based on more readily available information like soil texture are satisfactory. Saxton *et al*. (1986) presented the relationship between soil water content (%) and soil WP for different soil types. As an example (Figure 2-5), they showed that when soil WP is 0 MPa, soil moisture is between 20-30% for sandy loam and silty loam soil types respectively. Decreasing soil moisture down to 13% for the both soil types was almost equal to the soil WP of -1 MPa.

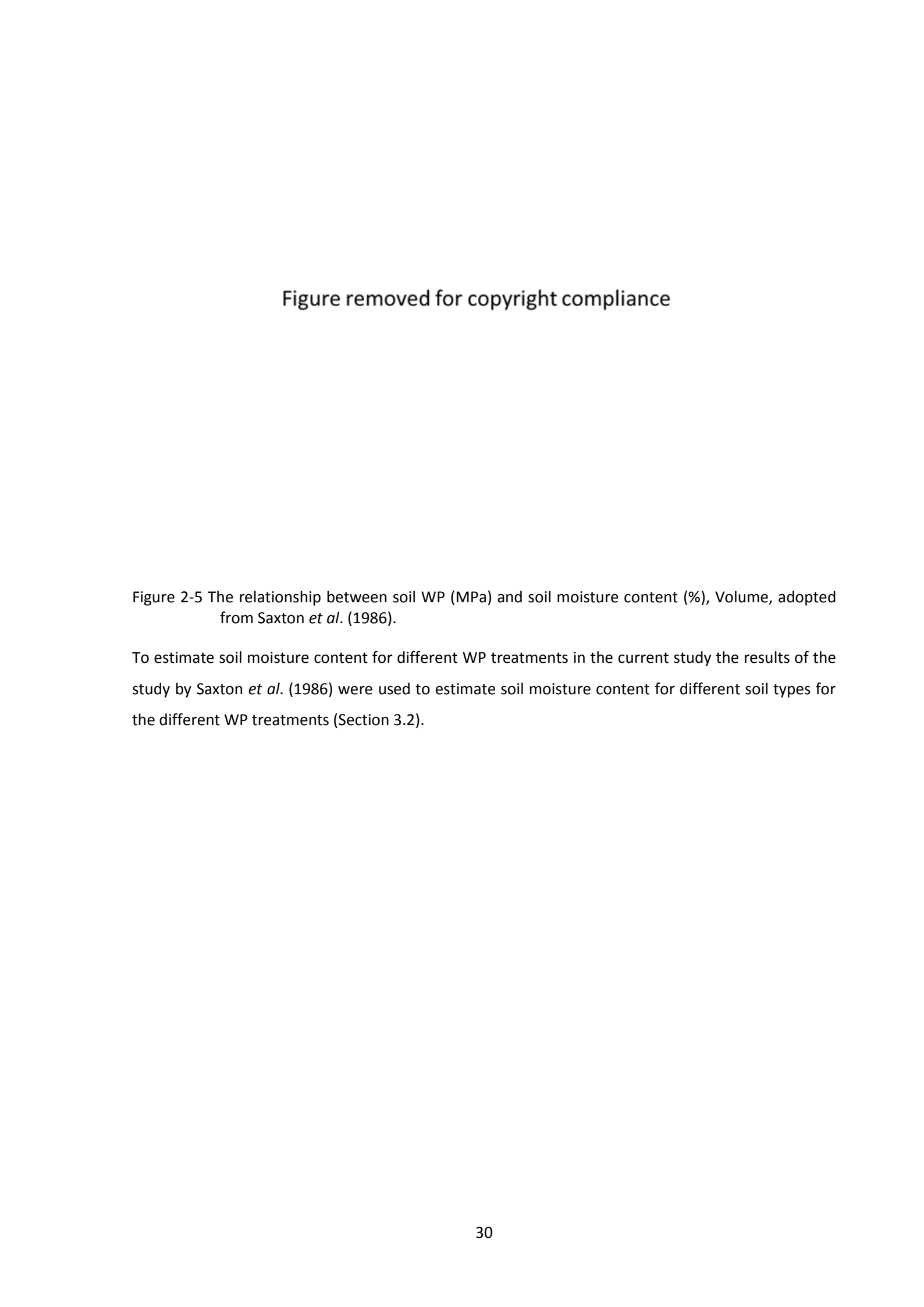


Figure 2-5 The relationship between soil WP (MPa) and soil moisture content (%), Volume, adopted from Saxton *et al*. (1986).

To estimate soil moisture content for different WP treatments in the current study the results of the study by Saxton *et al*. (1986) were used to estimate soil moisture content for different soil types for the different WP treatments (Section 3.2).

2.11 Conclusions

The following conclusions can be drawn based from this literature review:

• Temperature affects canopy expansion and photosynthetic rate resulting in variation in seasonal DM production within and among environments. Potential crop DM is the result of solar radiation interception and the efficiency with which it is converted into biomass. Disease and pests, moisture availability and nutrition are the other elements that affect the seasonal distribution of yield.

• Moisture stress reduces the amount of intercepted solar radiation due to its affect on canopy expansion, before photosynthesis is inhibited.

• The metabolic N pool decreases as a result of N deficiency. The decrease in biomass production frequently observed with N deficiency can arise from either a reduction in the radiation interception by the canopy, RUE or a combination of both factors.

• Water supply can be defined by the PAWC of the soil and water extraction rate by the root front moving down the soil profile. Effects of different PAWC on production of brome, cocksfoot, perennial ryegrass and tall fescue will be examined in the current study.

• Moisture stress can be quantified as transpiration demand in relation to supply. Yield reduction can be related to the effect of moisture stress on the fraction of radiation interception and photosynthetic rate.

• Moisture and temperature are the most important factors that affect germination rate and percentage and pasture establishment.

To answer the main research objectives of the current research, which are to understand moisture and N effects on dryland brome, cocksfoot, perennial ryegrass and tall fescue growth under summer dry conditions, two field experiments were used. Crops were grown in two soils which differed in their PAWC, at Ladbrooks and Ashley Dene. Then the relationships between yield and the major factors affect production including temperature, moisture and N could be quantified.

Since germination is the initial step for successful establishment of pastures, Chapter 3 investigates the combined effects of the most important factors (moisture and temperature) on seed germination of brome, cocksfoot, perennial ryegrass and tall fescue by defining a hydrothermal time model for each species.

Chapter 3 'Hydrothermal time' to quantify and model germination

3.1 Introduction

Seed germination is responsive to temperature (T), WP (WP), light and the quantity of accessible oxygen in the seedbed (Bewley and Black, 1994). Among these, WP and accumulated thermal units determine the germination rate and percentage (Baskin and Baskin, 1998).

In New Zealand, pasture sowing can occur at any time from spring to autumn, based on temperature and soil moisture, as well as pest pressures, weeds and other farm management decisions. Some pastures are sown in spring or early summer but 85% or more pastures are autumn sown (Wynn-Williams, 1982). This may result in germination/emergence failure of drought-sensitive species particularly when sown in to a warm and dry seedbed (Moot *et al*., 2003).

Understanding germination rate of pasture species under different conditions is important in terms of weed management. Weeds compete for light, moisture and nutrients and the slower germination and leaf area expansion rate of pasture seedlings, relative to weeds, means new pastures are prone to weed invasion (Palmer, 1982).

Understanding the combined effects of moisture and temperature on the percentage of seed germination and rate of germination for pasture species is necessary to develop appropriate strategies for timing and methods for pasture seeding and management in the establishment phase.

In this study, a hydrothermal time model (HTT) was used to predict seed germination and initial growth of brome, cocksfoot, perennial ryegrass and tall fescue seedlings under different combinations of T and WP. The results have implications for predicting germination and establishment under varied field conditions.

3.2 Materials and methods

Four pasture grass species were evaluated: pasture brome ('Bareno 9045D'), cocksfoot ('SFR36-009'), perennial ryegrass ('Stellar AR1') and tall fescue ('FinesseQ'). All were supplied by Seed Force Ltd., Christchurch, New Zealand. Seeds were incubated for at least 50 days in the dark, in constant controlled temperatures of 5, 10, 15, 20, 25, 30 and 35 °C and five WPs (0, -0.18, -0.37, -0.63 and -0.95 MPa). To have more accurate estimation of the ceiling temperature, all seeds species were also sown in distilled water (0 MPa) and incubated in 27.5 and 32.5 °C.

The solutions with different WPs were created using polyethylene glycol (PEG), as described by Hardegree and Emmerich (1990) and summarised in Equation 3-1:

Equation 3-1 $\Psi = 0.130[\mathrm{PEG}]^2T - 13.7[PEG]^2$

Where Ψ is the osmotic potential of a solution of PEG 8000 in water (MPa), [PEG] is the concentration of PEG 8000 in water (g PEG 8000 g H_20^{-1}) and T is the solution temperature in 0 °C. The following WPs were created: 0, -0.18, -0.37, -0.63 and -0.95 MPa.

Solution WPs were checked with an Osmometer (Wescor Inc, Logan, Utah, U.S.A.) to ensure that each solution had the intended WP at the beginning of the experiment. The osmotic potential was also checked every week for the 5, 10, 12, 15, 20 and 25 °C temperatures and every 4 days for the 30 °C and 35 °C treatments to ensure that it did not change due to evaporation during the experiment.

Experiments for each species used three replicates of a factorial combination of the temperatures and WPs. For each replicate, 50 grass seeds were dusted with Thiram (Tetramethylthiuram disulphide) to minimize fungal growth and placed onto Whatman No. 2 germination paper in 750 mm^2 plastic trays. This resulted in 150 seeds per WP/temperature combination. To initiate the experiment, 10 ml of the solution with the specified WP was added to each container to ensure that the WPs were unchanged during the experiment. After the seeds were placed in the germination containers, a plastic snap-top was secured and trays were placed in incubators at the specified temperatures.

Each container was randomly placed within the incubator and its location was changed systematically on a daily basis to reduce the potential for spatial variation in air temperature that could result from uneven air circulation in incubators.

Seeds were checked every 24 hours to count the number of germinated seeds in each replicate of all treatments. Seeds were considered to have germinated when the radicle protruded more than 2 mm from the seed coat (Ariefdjohan *et al*., 2004). At each measurement, germinated seeds were removed and the number of germinated seeds was recorded.

Due to unexpected results for brome seeds (Section 3.5) a part of the experiment was repeated. Germination of brome seeds under 0 and -0.18 MPa at 15, 20 and 25 °C was repeated three times. Since the results of the repeated experiments were consistent, only the data obtained from the first experiment is reported.

3.3 The effects of polyethylene glycol solution on seed germination

Polyethylene glycol solutions have been widely used to control WP in many seed germination studies (Young *et al*., 1983). However, in some cases there is concern that PEG solution-seed contact may decrease seed germination. Emmerich and Hardegree (1990) investigated the effects of PEG solution-seed contact on seed germination at 25 °C of four grass species: Sideoats grama (*Bouteloua curtipendula* (Michaux) Torrey), Buffelgrass (*Cenchrus ciliaris* L.), Lehmann lovegrass (*Eragrostis lehmanniana* Nees), and Kleingrass (*Panicum coloratum* L.). A control WP system (using filter paper and a cellulose membrane) was used for seed germination which controlled solution-seed contact of PEG (8000 mol. wt.). They showed that Buffelgrass and Lehmann Lovegrass germination and germination rate were unaffected by PEG solution-seed contacts when they were sown under different WPs, ranging from -1.62 to 0 MPa. However, final germination and germination rate of Sideoats grama and Kleingrass showed a reduction without PEG solution contacts on the cellulose membrane. The reduction was ascribed to the hydraulic conductivity at the seed water-membrane contact point which was affected by PEG solution WP and seed size and shape. The high ratio of PEG solution volume to filter paper (>12 L kg^{-1}) reduced the change in WP from PEG exclusion related to filter paper substrates. Therefore, it was concluded that PEG solution-seed contact does not decrease seed germination.

3.4 Data analysis

One assumption in regression analysis is that the data are independent (Hay *et al*., 2014). Calculating the cumulative number of germinated seeds does not satisfy this assumption. Seed germination experiments could be designed with successive observations made on independent samples of the seeds. This would create independent observations and regression analysis would then be completely valid. For example, a single sample of seeds would be required to be sown under each temperature treatment. For each specific observation time point, each Petri dishes would be observed once and then discarded.

In this way, the data would meet the assumption of linear regression analysis and it would be possible to use a probit analysis approach to fitting germination progress models like thermal time, hydrotime and/or hydrothermal time. However, had this approach been followed, considerably more seeds would have been required and the experiment would have required more time and been more expensive. Bradford (1995) reported that '. . . proper application of probit analysis requires independent measurements at each time point (Finney, 1971)'. However, he concluded that the observed germination data would be the same whether collected from dependent or independent samples, and thus that a probit analysis method was valid.

Probit-based models have been used in seed biology to describe the rate of loss of viability due to seed ageing and germination progress over time in response to environmental variables (e.g. temperature, moisture). This model connects a proportional response variable to a temporal explanatory variable and assumes that the times to response are normally distributed through the seed population. In germination experiments, data are usually recorded as a cumulative number of germinated seeds for each sample when the observation is successive (Hay *et al*., 2014). In this study, the number of germinated seeds in every replicate of each treatment was recorded as cumulative. The daily cumulative number of germinated seeds was used to calculate hydrothermal time parameters.

Plotting different percentiles of seed germination versus temperature, showed a linear relationship. Linear regression lines were fitted to the data using Genstat 16.1. [Copyright 2013, VSN International Ltd.]. Regression lines converged to the same point (± s.e.) at the temperature axis (X axis) which is reported as the base temperature (Tb) for each species.

The maximum number of germinated seeds for each species under the optimum conditions (T= To for the specific species and WP of 0 MPa) is called the control viability coefficient. Germination percentiles of all treatments were divided in to the control viability coefficient.

Model parameters including the hydrotime constant θH (MPa °d), hydrothermal time constant θHT (MPa °d), seed base WP Ψb (g) (MPa) and standard deviation of the base WP (σ Ψb (g) (MPa)) were calculated using generalized linear models (GLMs) with a probit (inverse of cumulative normal distribution) link function, using Genstat 16.1. The binomial error distribution of the dependent variable was taken into account. Predicted germination percentiles are presented as percentages in Section 3.5.

3.4.1 Estimation of the hydrothermal model parameters

To fit the hydrothermal time model as a GLM, data were entered as the quantile of germinated seeds daily (number of germinated seeds/number of sown seeds) for the dependent variable (g), with three explanatory variables, WPs (Ψ)s, the inverse of the time to germination (t^{-1}) and temperature (T). Individual seeds, in each treatment were given a percentile number, starting from 0.02 (1/50) for the fastest, and 0.04 (2/50) for the second fastest germinated seed etc. This was then divided by the control viability coefficient (Section 3.4).

The probit analysis provided estimates for the three unknown parameters in the GLM from which were calculated hydrotime parameters θHT = –ß2/ß1, Ψb (50) = –ß0/ß1 and σΨb = 1/ß1 from which germination could be predicted (Gr pred) based on the model parameters of Equation 3-2.

Equation 3-2 $\mathrm{GR\ pred} = [\beta 0 + \beta 1\Psi + \beta 2\ (\mathrm{t}^{-1}(\mathrm{T} - \mathrm{Tb}))^{-1}$

Since the base temperature was nearly constant among different germination percentiles, a single Tb was defined for each species, using a linear regression fitted to GR (50) versus temperature data at sub-optimal temperatures for WP at 0 MPa (Section 3.4). (t^{-1}(T-Tb) was then calculated by using the inverse of the time to germination (t^{-1}) for each germination percentile for each temperature (T) treatment.

In Equation 3-2, if the base WPs of all seed percentiles (Ψb (g)) in a specific seedlot are normally distributed (e.g. Dahal and Bradford, 1994), then the probit function is able to calculate the standard normal deviate (z) for a specified cumulative P.

To do this probit(g) was used to calculate the standard normal deviation (z) of every individual seed. Probit(g) could be mathematically described as:

Equation 3-3 $\mathrm{probit\ (g)} = [\Psi \mathrm{b\ (g)} - \Psi \mathrm{b\ (50)}]/\sigma\Psi \mathrm{b}$

Since the WPs are normally distributed, Ψb (50) in this equation is the mean Ψb (g). The predicted Ψb (g)s were calculated in Equation 3-4:

Equation 3-4 $\Psi \mathrm{b\ (g)pred} = \Psi - (\theta \mathrm{HT}/[(\mathrm{T} - \mathrm{Tb})\ \mathrm{t\ (g)}])$

3.4.2 Germination model used at supra-optimal temperatures

In the model described by Alvardo and Bradford, the upwards adjustment to Ψb (g) at supra-optimal temperatures is predicted by Equation 3-5:

Equation 3-5 $\Psi \mathrm{b\ (g)}' = \Psi \mathrm{b\ (g)} + \mathrm{k}[\mathrm{T} - \mathrm{To}]$

In this equation, Ψb (g)′, is the adjusted base WP at supra-optimal temperatures for the g^{th} percentile of a specific seed population and K is the slope of the relationship between ψb (g) and T in the supra-optimal range if To is a constant.

3.5 Results

The maximum germination percentages of cocksfoot, tall fescue, perennial ryegrass and brome were 82%, 90%, 93% and 98%, respectively, at 0 MPa WP when temperature (T) was 20 °C. These numbers defined the control viability coefficient. The final germination percentage for each species was divided by the control viability coefficient for all treatments (Section 3.4).

Under non-limiting water conditions, all grass species had their lowest maximum final germination (%) at 5 °C and 35 °C. Brome seed germination rate and the final germination percentages were unaffected

by decreasing WP from 0 to -0.18 MPa, when 15≤T≤30 °C. For the other species, determining germination rates (1/tg) for the 10^{th}, 30^{th}, 50^{th} and 70^{th} percentile of the seed germination against WPs showed a linear reduction in germination rate as WP decreased from 0 to -0.95 MPa.

3.5.1 Thermal time response of seeds under non-limiting water conditions

Germination percentages over time for all species are shown in Figure 3-1. At 5 °C, perennial ryegrass started germination after 12 days and almost 35% of the seeds germinated after 23 days. Cocksfoot and tall fescue required 22 and 30 days to initiate germination at 5 °C. The maximum number of germinated seeds for tall fescue was almost 15% at the same temperature. Brome seeds did not germinate at 5 °C (Figure 3-1 A–D).

As temperature increased (within the sub-optimal range), germination rate and final percentage increased for all species. At 10 °C, all species began germination in 7 to 9 days and final germination percentage of cocksfoot, brome and perennial ryegrass was ≥90% with 78% for tall fescue. At 15 °C, brome began germination 4 days after sowing, tall fescue and perennial ryegrass 5 days and cocksfoot 7 days (Figure 3-1 A–D). Final germination percentage was ≥90% for all species at 15 °C. Cocksfoot, tall fescue and brome began germination 3 days after sowing whereas perennial ryegrass germination started 2 ½ days after sowing at 20 °C and 2 days after sowing at 25 °C.

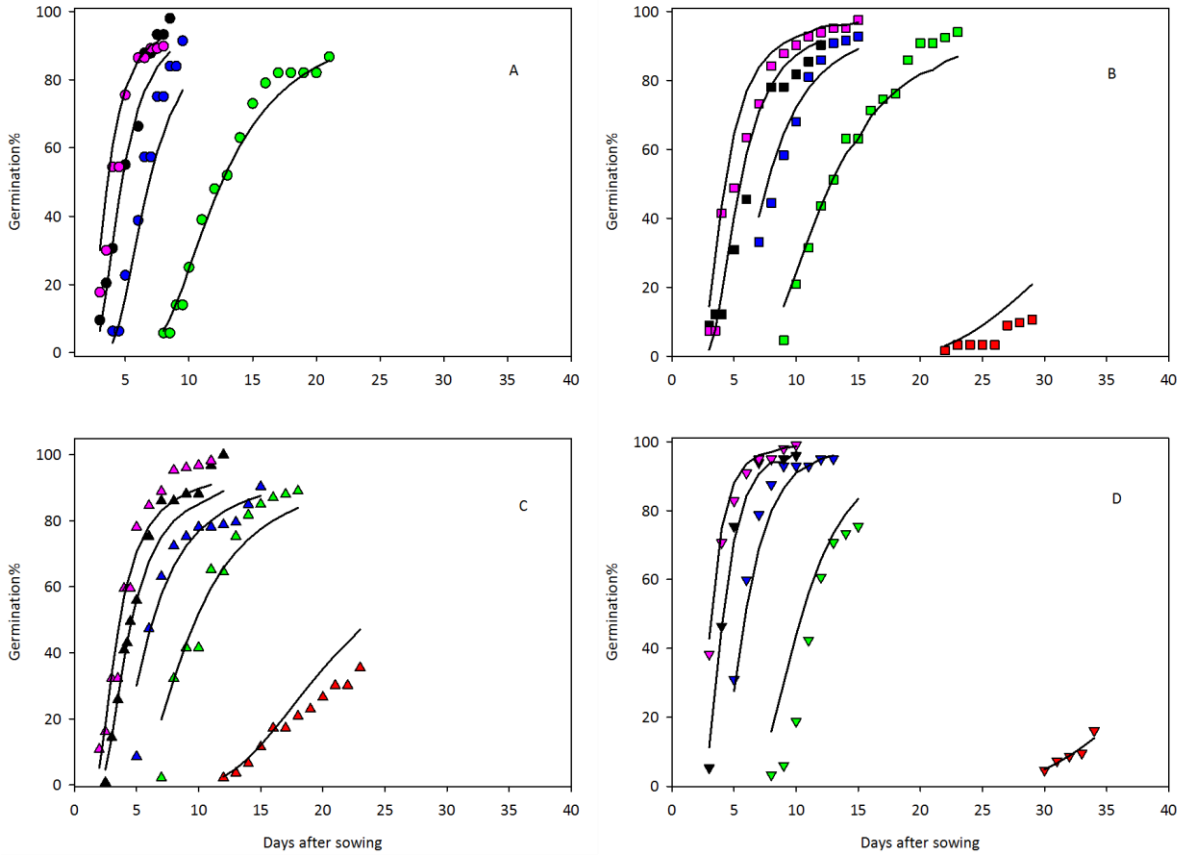


Figure 3-1 Germination percentages of A) brome (●), B) cocksfoot (■), C) perennial ryegrass (▲) and D) tall fescue (▼) seeds at 0 MPa, under different temperatures (● = 5, ● = 10, ● = 15, ● = 20 and ● = 25 °C). The symbols are the actual data, and the lines are the time courses predicted by hydrothermal time model (Section 2.10).

Figure 3-2 shows the number of days after sowing required for 50% of the final seed germination at each temperature in distilled water (0 MPa). Across all temperatures there was an exponential decline in the number of days to 50% of germination for all species as temperature increased in the sub-optimal range (Figure 3-2 A-D). Between 20 and 25 °C all species required ~5 days to obtain 50% of the final germination percentage.

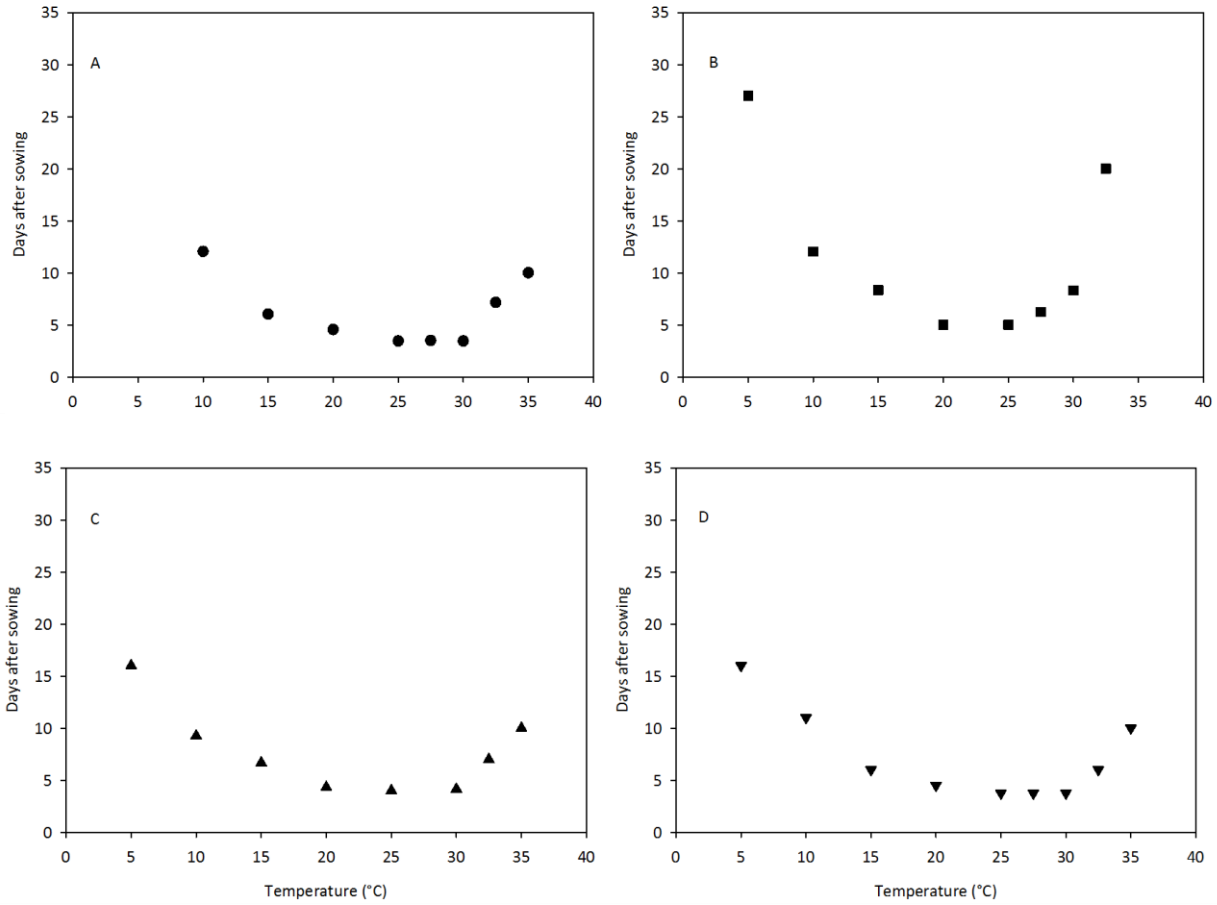


Figure 3-2 Time to 50% of final seed germination of A) brome (●), B) cocksfoot (■), C) perennial ryegrass (▲) and D) tall fescue (▼) seeds under different temperatures (5-35 °C) in water (0 MPa).

Using the inverse of time (1/day or d^{-1}) to calculate the rate of germination (Equation 2-8) and thermal time (Tt) at sub- (θT1) and supra- (θT2) optimal range of temperatures (Equation 2-7 and Equation 2-9), provided an estimate of the cardinal temperatures for each species (Figure 3-3). Ceiling temperatures (Tcs) were different for each percentage of germinated seeds. Ceiling temperatures were highest for the fastest germinating seeds (i.e. the lowest percentages in the population); Tc50 was 36 °C for brome, 35.3 °C for cocksfoot and 39 °C for tall fescue and perennial ryegrass. The lowest thermal time requirement for 50% of final germination (Tt50) was 90 °Cd for perennial ryegrass and the highest was 114 °Cd for cocksfoot.

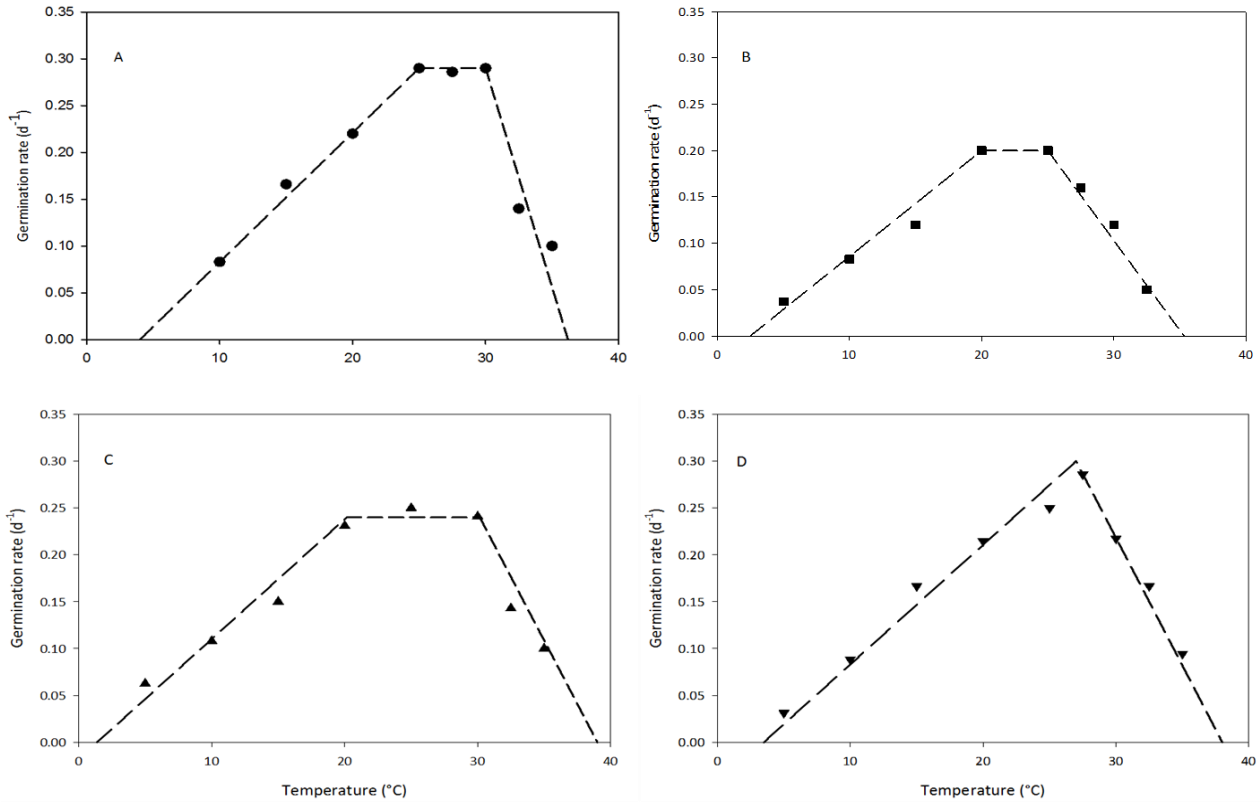


Figure 3-3 Germination rate (GR (50)) of A) brome (●), B) cocksfoot (■), C) perennial ryegrass (▲) and D) tall fescue (▼) seeds under different temperatures (5-35 °C) in water (0 MPa). Dashed lines (– – –) are linear regressions fitted to data at sub- and supra- optimal temperatures.

Under the specified optimum temperature/temperatures for each species, germination rate of brome (0.30 d^{-1}) was higher ($P<0.01$) than for the other grasses. Maximum germination rate for tall fescue was 0.28 d^{-1} at To = 27.5 °C, compared with 0.25 d^{-1} for perennial ryegrass when 20≤To≤30 °C. Cocksfoot had the lowest maximum germination rate (0.20 d^{-1}) at its optimum temperature range (25≤To≤30 °C).

3.5.2 Germination rate and final germination percentage response to temperature

Species showed different responses to increasing temperature above 25 °C in terms of both germination rate and the final germination percentage. Final germination of perennial ryegrass and tall fescue was ≥87% at 25 and 30 °C. Germination rate of brome was still at the optimum range when T = 30 °C, but the final germination was 80% when temperature increased from 25 to 30 °C. For tall fescue, germination rate decreased at 30 °C (supra-optimal range), but the final germination was still 87%. Final germination of cocksfoot decreased rapidly from 80% at 25 °C to 37% at 30 °C. Final germination of tall fescue, brome and perennial ryegrass was only 10%, 15% and 15% at 30 °C, 32.5 °C and 35 °C. These were therefore defined as supra-optimal temperatures for all species. Cocksfoot seeds did not germinate at 35 °C.

3.5.3 Germination responses to different water potentials by time (Hydrotime)

For all species at any constant temperature, decreasing the ambient WP caused a delay in achieving the final germination percentage and/or decreased the germination rate compared with the germination rate in water (0 MPa). Figure 3-4 shows the germination time course of all grasses at different WPs (0, -0.18, -0.37, -0.63 and -0.95 MPa) at 20 °C. The only exception was brome at -0.18 MPa (Figure 3-4 A). In this case, decreasing WP to -0.18 MPa did not cause a reduction in germination rate and final germination percentage compared with the germination rate in water (0 MPa).

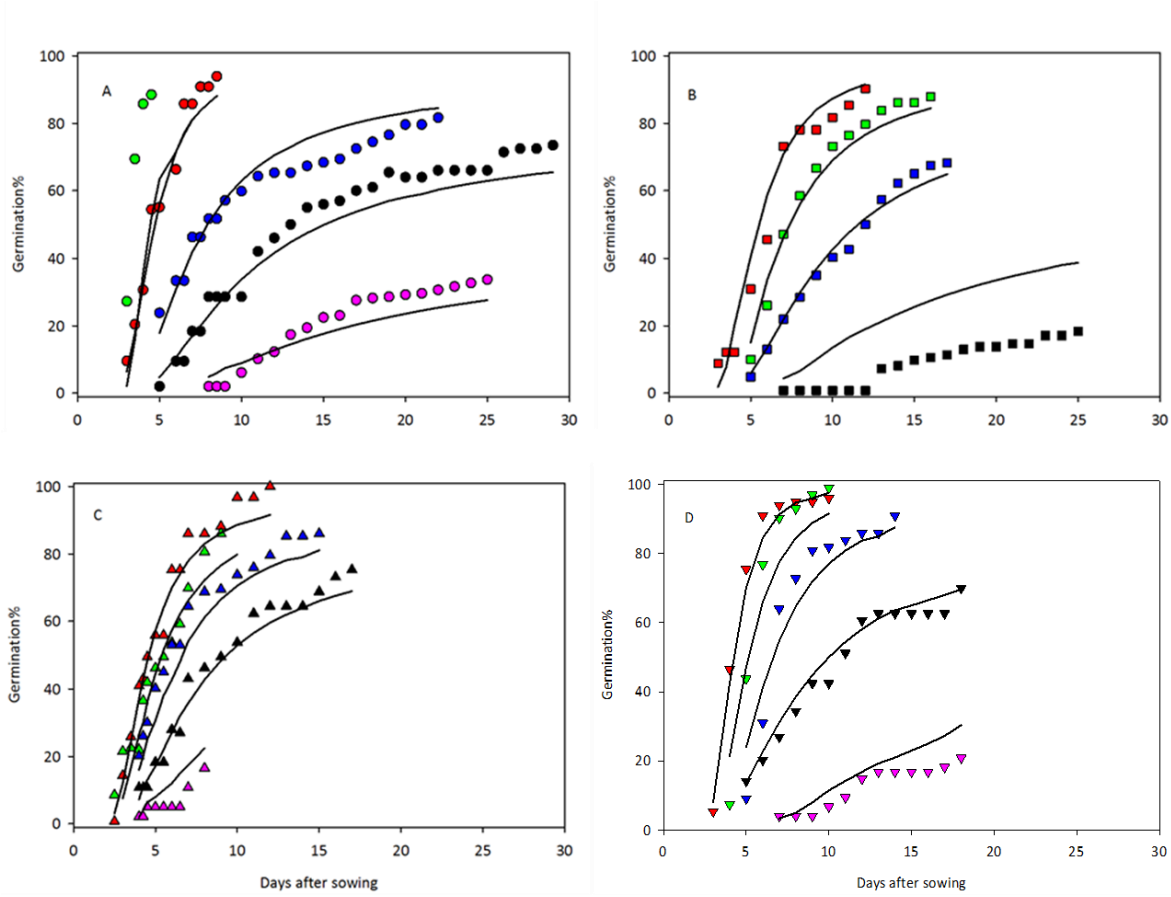


Figure 3-4 Germination time courses (day) of A) brome (●), B) cocksfoot (■), C) perennial ryegrass (▲) and D) tall fescue (▼) seeds imbibed at different WP treatments (0 (●), -0.18 (●), -0.37 (●), -0.63 (●) and -0.95 (●) MPa) at 20 °C. The symbols are the actual data, and the lines are the time courses predicted by the hydrothermal time model using the values shown in Tables 3-1 to 3-4. Normal distributions showing the relative frequencies of ψb (g) values at each temperature. The median or ψb (50) values are also presented in Tables 3-1 to 3-4.

Table 3-1 Parameters of the hydrothermal time model characterizing germination of brome seeds imbibed at four WPs (0, -037, -0.63 and -0.95 MPa) at sub- and supra-optimal temperatures.

Brome

Sub-optimal HTT model					**Cardinal temperatures (°C)**		
Temperatures (°C)	**ΘH (MPa d^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**Tc**	**Tb**	**To**
10	58.2	-0.80	0.27	0.98	36	4±0.50	25-30
15	58.8	-0.85	0.28	0.97			
20	71.3	-0.96	0.37	0.94			
25	68.5	-0.83	0.36	0.97			
Temperatures (°C)	**ΘHT (MPa °Cd^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**RMSE**		
5-25	69.3	-0.90	0.34	0.92	0.009		
Supra-optimal HTT model							
Temperatures (°C)	**ΘH (MPa d^{-1})**	**Ψb (50) ′ (MPa)**	**σΨb**	**R^2**	**RMSE**	**k (MPa °C^{-1})**	
30	62.2	-0.68	0.40	0.94	0.024	0.01	

Table 3-2 Parameters of the hydrothermal time model characterizing germination of cocksfoot seeds imbibed at five WPs (0, -0.18, -037, -0.63 and -0.95 MPa) at sub- and supra-optimal temperatures.

Cocksfoot

Sub-optimal HTT model					**Cardinal temperatures (°C)**		
Temperatures (°C)	**ΘH (MPa d^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**Tc**	**Tb**	**To**
10	58.2	-0.60	0.24	0.93	35.3	2.5±0.20	20-25
15	83.1	-0.84	0.27	0.93			
20	53	-0.59	0.22	0.93			
Temperatures (°C)	**ΘHT (MPa °Cd^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**RMSE**		
5-20	63	-0.66	0.26	0.91	0.009		
Supra-optimal HTT model							
Temperatures (°C)	**ΘH (MPa d^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**			
25	-	-0.047	0.45	0.45			
30	-	-0.001	0.31	0.41			
Temperatures (°C)	**ΘHT (MPa °Cd^{-1})**	**Ψb (50) ′ (MPa)**	**σΨb**	**R^2**	**RMSE**	**k (MPa °C^{-1})**	
25							

Table 3-3 Parameters of the hydrothermal time model characterizing germination of perennial ryegrass seeds imbibed at five WPs (0, -0.18, -037, -0.63 and -0.95 MPa) at sub- and supra-optimal temperatures.

Perennial ryegrass

Sub-optimal HTT model					**Cardinal temperatures (°C)**		
Temperatures (°C)	**ΘH (MPa d^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**Tc**	**Tb**	**To**
5	79	-0.8	0.55	0.74	39	1.5±0.25	20-30
10	115	-1.3	0.5	0.76			
15	118	-1.3	0.7	0.84			
20	105	-1.3	0.52	0.88			
25	75	-0.95	0.32	0.99			
Temperatures (°C)	**ΘHT (MPa °Cd^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**RMSE**		
5-25	90	-1.1	0.44	0.92	0.07		
Supra-optimal HTT model							
Temperatures (°C)	**ΘH (MPa d^{-1})**	**Ψb (50) ′ (MPa)**	**σΨb**	**R^2**	**RMSE**	**k (MPa °C^{-1})**	
30	60	-0.70	0.33	0.74	0.11	0.24	

Table 3-4 Parameters of the hydrothermal time model characterizing germination of tall fescue seeds imbibed at five WPs (0, -0.18, -037, -0.63 and -0.95 MPa) at sub- and supra-optimal temperatures.

Tall fescue

Sub-optimal HTT model					**Cardinal temperatures (°C)**		
Temperatures (°C)	**ΘH (MPa d^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**Tc**	**Tb**	**To**
10	83	-1.13	0.30	0.77	39	3.5±0.45	27.5
15	90	-1.06	0.28	0.97			
20	70.4	-1.02	0.24	0.95			
25	66.1	-0.95	0.29	0.99			
Temperatures (°C)	**ΘHT (MPa °Cd^{-1})**	**ψb (50) (MPa)**	**σΨb**	**R^2**	**RMSE**		
5-25	69.3	-0.90	0.34	0.92	0.008		
Supra-optimal HTT model							
Temperatures (°C)	**ΘH (MPa d^{-1})**	**Ψb (50) ′ (MPa)**	**σΨb**	**R^2**	**RMSE**	**k (MPa °C^{-1})**	
						0.042	
30	54.12	-0.68	0.22	0.91	0.078		

Determining germination rates (1/tg) for the 10^{th}, 30^{th}, 50^{th} and 70^{th} percentile of the seed germination against WPs showed a linear reduction in germination rate as WP decreased from 0 to -0.95 MPa

(Appendix A). At the sub-optimal range specified for each species, ψb (50) and σψb values were not differ across temperatures (Tables 3-1 to 3-4). At supra-optimal temperatures, increasing temperature above To caused an increase in the base WP, which resulted in increased hydro-time requirements (Equation 2-11). However, this is not reflected in the ΘH values reported for supra- optimal temperatures in Tables 3-1 to 3-4. This is because, at supra-optimal temperatures, germination only occurred under a number of WP treatments. As an example, at supra- optimal temperatures specified for tall fescue, germination only occurred at 0, -0.18 and -0.37 MPa and no germination occurred at -0.63 or -0.95 MPa. The seeds germinated rapidly under moist conditions (0 MPa) and when WP= -0.18 MPa at supra-optimal temperatures. However, for the rest of the population, decreasing WP below -0.18 MPa caused a progressive rise in Ψb (g) at supra-optimal temperatures which caused no germination to occur in this case. Therefore, ΘHs reported in Tables 3-1 to 3-4 only account for a limited number of WP treatments.

3.5.4 Combined effect of the ambient water potentials and temperatures on germination rate of 50% of the seeds (GR (50))

By definition, for all species the fastest rate of germination occurred at the optimum temperature/ temperatures in water (0 MPa). The response surface diagrams display the effect of decreased WP at each temperature (Figure 3-5 A-D). The results show that, decreasing WP within the range of treatments caused a decrease in the germination rate of all species with the exception of brome under -0.18 MPa.

Brome

The maximum germination rate for brome was 0.30 d^{-1} which was higher ($P<0.01$) than the other species at its optimum temperature (30 °C) when WP was 0 MPa (Figure 3-5 A). When 15 ≤T≤ 30 °C and WP was -0.18 MPa the germination rate (and final germination percentage) of brome did not decrease. At the same WP range of temperature (15≤T≤30 °C), germination rate of brome was >0.20 d^{-1} even when WP was decreased to -0.63 MPa. When T≤15 °C, germination rate of brome was 0.05≤GR (50)≤0.15 d^{-1} across all WPs treatments.

Cocksfoot

Germination rate of cocksfoot was slower ($P<0.01$) than other species (Figure 3-5 B). The maximum GR (50) for cocksfoot was between 0.16 d^{-1} and 0.20 d^{-1} which occurred at 20≤T≤25 °C and 0≥WP≥-0.18 MPa. At 10≤T≤20 °C, GR (50) of cocksfoot was 0.11 d^{-1}≤GR (50)≤0.05 d^{-1} under -0.37 MPa. When temperature was between 25 and 35 °C, under decreased WP of -0.37 MPa and below that, cocksfoot seeds germination rate was 0 to 0.05 d^{-1} (Figure 3-5 B).

Perennial ryegrass

When 20≤T≤30 °C, a mild moisture stress (-0.18 MPa) caused a maximum of 0.04 d^{-1} reduction in GR (50) compared with 0 MPa. Decreasing WP to -0.63 MPa, at the same range of temperatures, caused a maximum of 0.07 d^{-1} reduction in GR (50) compared with 0 MPa (Figure 3-5 C).

Tall fescue

At 25 °C and 30 °C, GR (50) was 0.21 to 0.25 d^{-1} under both -0.18 MPa and -0.37 MPa (Figure 3-5 D). When temperature was 25 °C, decreasing WP to -0.63 MPa, caused a 0.07 d^{-1} decrease in GR (50).

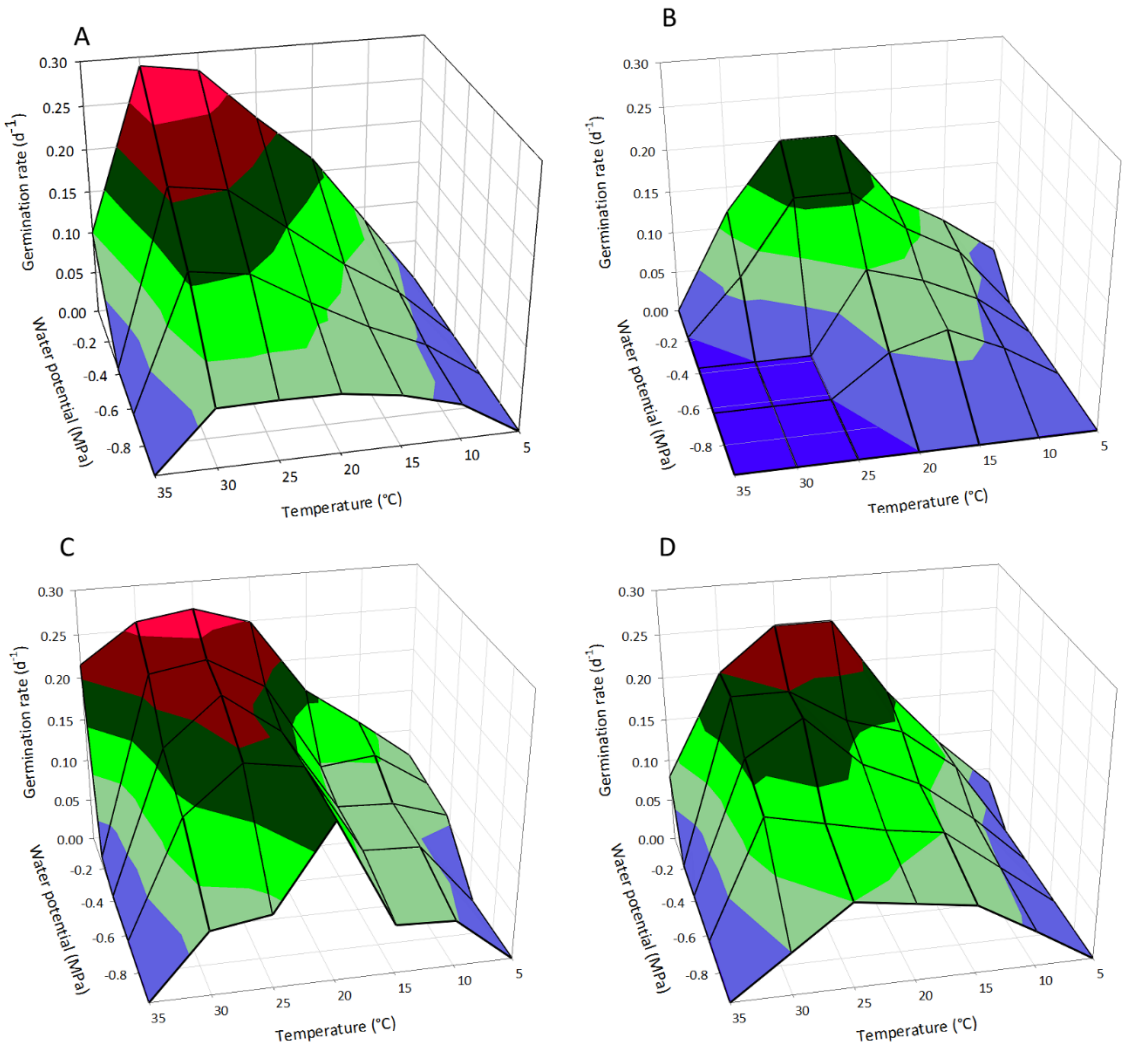


Figure 3-5 Rate to achieve 50% of final germination (GR (50) for A) brome, B) cocksfoot, C) perennial ryegrass and D) tall fescue. Colours show germination rates of 0 d^{-1}, 0.01-0.05 d^{-1}, 0.06-0.10 d^{-1}, 0.11-0.15 d^{-1}, 0.16-0.20 d^{-1}, 0.21-0.25 d^{-1} and 0.26-0.30 d^{-1}.

3.5.5 Combined effect of the ambient water potentials and temperatures on final germination percentage

In most cases decreasing WP (more negative) caused a reduction in germination rate, (except for brome when WP = -0.18 MPa. The interacting effects of temperature and WP on the final germination percentage of brome, cocksfoot, perennial ryegrass and tall fescue are shown as response surface matrices in Figure 3-6. In some cases, under decreased WPs, final germination percentage remained unchanged (or changed less than 10%) relative to the optimum conditions. The final germination percentage of brome was still ≥80% when 10≤T≤20 °C and WP was -0.18 MPa. Also ≥80% of tall fescue seeds germinated when 15≤T≤30 °C under no moisture stress (0 MPa) and once WP was -0.18 MPa and -0.37 MPa (Figure 3-6 D). Cocksfoot showed ≥80% final germination when temperature was 10≤T≤25 °C and moisture was not limited (0 MPa). The final germination of cocksfoot was ≥80% at 15 and 20 °C, when WP was -0.18 MPa (Figure 3-6 B). Perennial ryegrass final germination was ≥80% under a broader range of temperatures (10≤T≤30 °C) when WP was 0 MPa. Even when WP was -0.37 MPa, perennial ryegrass seeds achieved ≥80% final germination when 20≤T≤25 °C (Figure 3-6 C).

When 10≤T≤25 °C, more than 50% of the brome seeds germinated even when WP was -0.63 MPa. At 30 °C, 80% and 63% of the brome seeds germinated when WP was 0 and -0.37 MPa. However, at the same temperature, only 40% of the brome seeds germinated when WP decreased to -0.63 MPa. Maximum final germination of brome at -0.95 MPa was 28% at 25 °C but the minimum was only 4% at 30 °C. Final germination for brome was ≤15% at 35 °C when WP was 0 MPa. (Figure 3-6 A).

More than 50% of perennial ryegrass seeds germinated when 10≤T≤30 °C even under decreased WPs of -0.18 and -0.37 MPa. Increasing moisture stress to -0.63 MPa caused a reduction in final germination (48% and 42%) when temperatures were 10 °C and 30 °C. Final germination of perennial ryegrass was still ≥50% when WP= -0.63 MPa was applied at 15≤T≤25 °C. Maximum final germination under WP= -0.95 MPa was 46% and 40% when temperature was 15 and 10 °C respectively (Figure 3-6 C).

Cocksfoot, was relatively sensitive to variation in both temperature and WP. More than 50% of cocksfoot seeds germinated under a narrow range of 10≤T≤20 °C when 0≥WP≥-0.37 MPa. Applying severe moisture stress (-0.63 MPa) caused a rapid decline in the final germination of cocksfoot at all temperatures. Maximum final germination was 21% at 15 °C at -0.63 MPa. Only at 15 °C, 8% of cocksfoot seeds germinate when WP was -0.95 MPa. No cocksfoot seeds germinated at 35 °C (Figure 3-6 B).

When 10≥T≥30 °C, ≥50% of tall fescue seeds germinated under moderate (-0.37 MPa) moisture stress. At 30 °C also, final germination was ≥50% when moderate stress (-0.37 MPa) was applied. When 10≤T≤25 °C, more than half of tall fescue seed germinated even under severe moisture deficit (-0.63

MPa). Only 12% and 4% of tall fescue seeds germinated when WP was -0.63 MPa and -0.95 MPa at 30 °C. Maximum final germination of tall fescue at severe moisture stress (-0.95 MPa) was 28% at 25 °C. Tall fescue seeds germination was 10% at 35 °C when moisture was not limiting (0 MPa) (Figure 3-6 D).

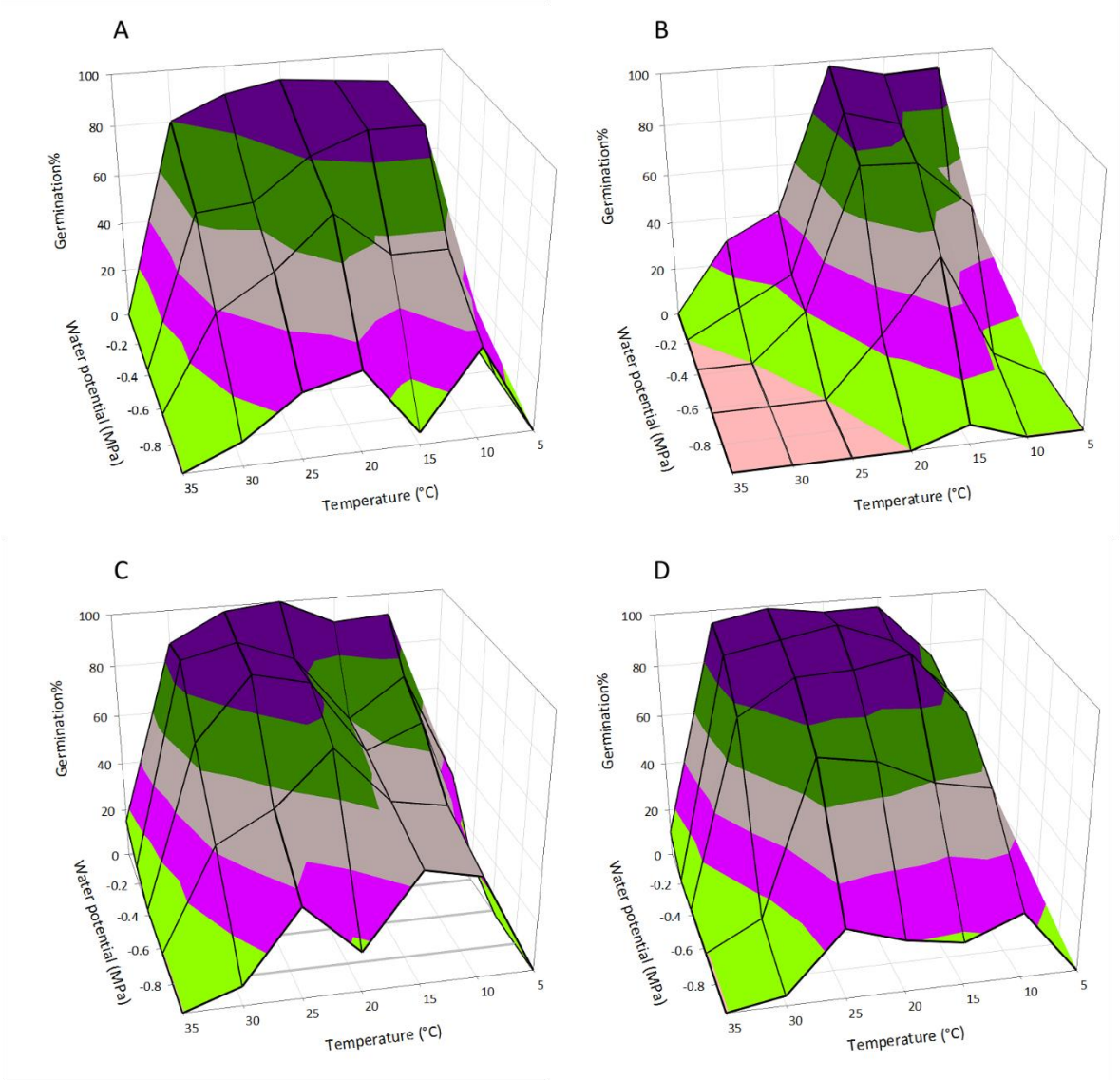


Figure 3-6 Final germination percentage of A) brome, B) cocksfoot, C) perennial ryegrass and D) tall fescue seeds under different WPs (0 to -0.95 MPa) and temperatures (5-35°C). Colours show germination percentage of (0%), (1-20%), (21-40%), (41-60%), (61-80%), (81-100%).

3.5.6 Hydrothermal time model of germination

Estimated hydrothermal model parameters at sub- and supra-optimal temperatures for each species are presented in Tables 3-1 to 3-4. The HTT model predictions of germination for sub-optimal temperatures were reasonably accurate ($R^2 \geq 0.91$). The estimated HTT constant at sub-optimal temperatures (5-25 °C) was 69.3 MPa °Cd^{-1} for brome and tall fescue and was lower than 90 MPa °Cd^{-1} for perennial ryegrass, which was due to the relatively lower (more negative) base WP of

perennial ryegrass (Equation 2-12). ΘHT at sub-optimal temperatures for cocksfoot (10≤T≤20 °C) was 63 MPa °Cd^{-1}. However, this cannot be compared with ΘHT for brome, perennial ryegrass and tall fescue since they had a wider range of sub-optimal temperatures (Tables 3-1 to 3-4). Comparing hydrotime constants at each specific temperature presented in Tables 3-1 to 3-4 shows a relatively higher (more positive) base WPs for cocksfoot under its specific range of sub-optimal temperatures (10≤T≤20 °C). Hydrotime constants (ΘH) are not presented for 5 °C since cocksfoot, tall fescue and brome did not germinate at decreased WPs at this temperature. Only perennial ryegrass germinated at decreased WP (-0.18 MPa) when temperature was 5 °C.

3.5.7 HTT model predictions of germination behaviour

Figure 3-7 shows the actual seed base WPs (Ψb (50)act) for the 50th percentile versus temperature, calculated from data from different WP treatments. The values of Ψb (50) act for different WPs are plotted against the predicted seed base WP (Ψb (50)pred) of the HTT model (Tables 3-1 to 3-4). At sub-optimal temperatures, the seed base WP of perennial ryegrass was much lower (-1.1 MPa) than for cocksfoot (-0.7 MPa), and was lower than brome (-0.87 MPa) but was similar to tall fescue (-1.05 MPa).

Under supra-optimal conditions, Ψb (50)act increased towards zero with increasing temperature above the optimum for all species, as described by Equation 3-5. For brome, there was some bias from the model once WP was -0.18 MPa at 15 and 20 °C. At 15 °C cocksfoot germination was more than the predicted values by the HTT model when WP was -0.63 MPa. Therefore Ψb (50)act was less accurately predicted Ψb (50) by the model. Since cocksfoot germination did not achieve 50% at 25 °C, prediction of Ψb (50) by model was not possible. This seems inconsistent with Figures 3-5 and Figure 3-6 where both final germination percentage and germination rate of cocksfoot declined more severely under dry and warm conditions than did brome, perennial ryegrass and tall fescue.

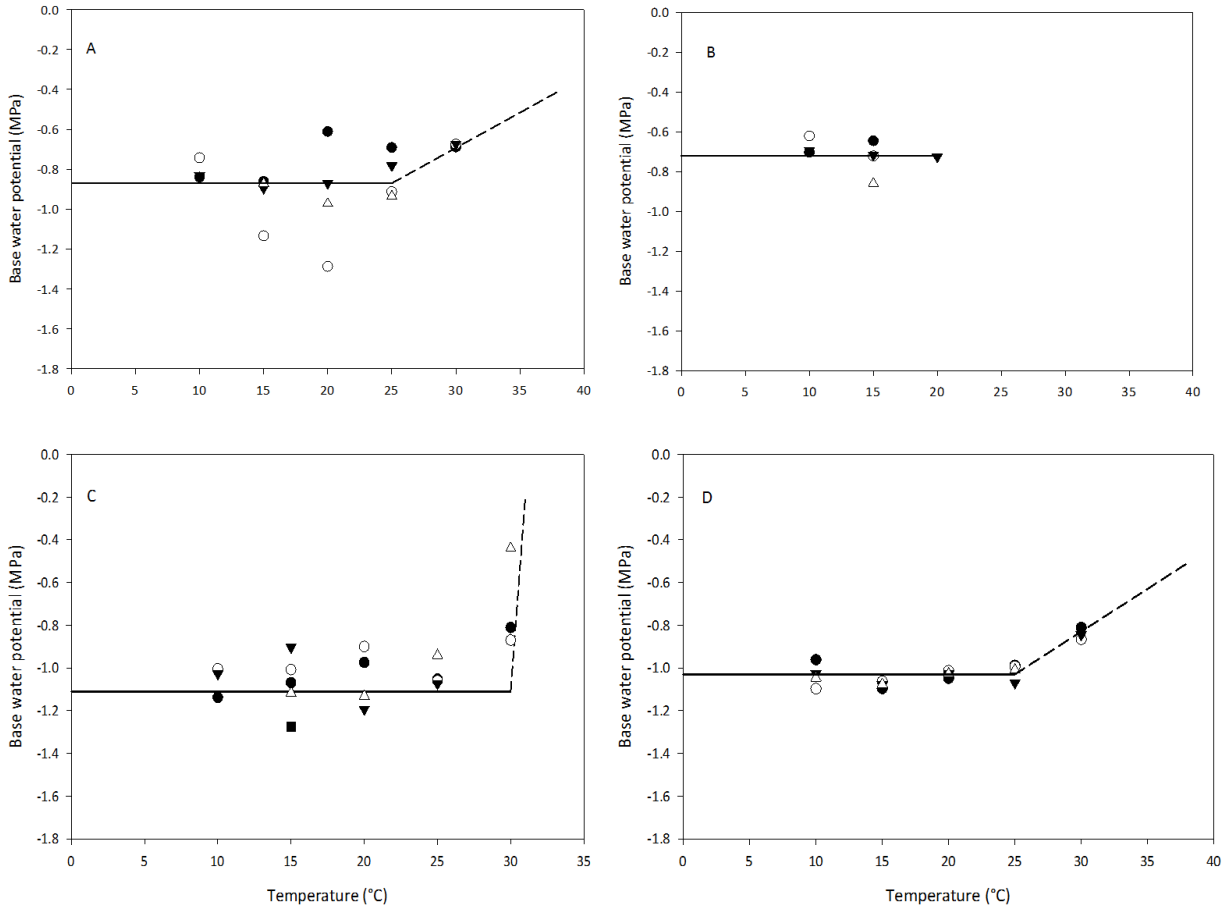


Figure 3-7 Actual base WPs (Ψb (50)act) against temperatures in A) brome, B) cocksfoot, C) perennial ryegrass and D) tall fescue. The symbols are Ψb (50)act under 0 (●), -0.18 (○), -0.37 (▼), -0.63 (△) and -0.95 (■) MPa. The horizontal lines (where T≤To) are Ψb (50)pred [= -0.90, -0.66, -1.1 and -0.90 MPa respectively] predicted by the HTT model. The diagonal lines (where T>To) have a slope of k = 0.10, 0.24 and 0.20 for brome, perennial ryegrass and tall fescue respectively.

A comparison of predicted versus actual germination percentile is shown for all species for sub-optimal (Figures 3-8) and supra-optimal (Figures 3-9) temperatures. In sub-optimal temperatures, some biases are shown for brome at 10 °C and 15 °C, cocksfoot at 15 °C, and 5 °C and for tall fescue at 5 °C (Figure 3-8).

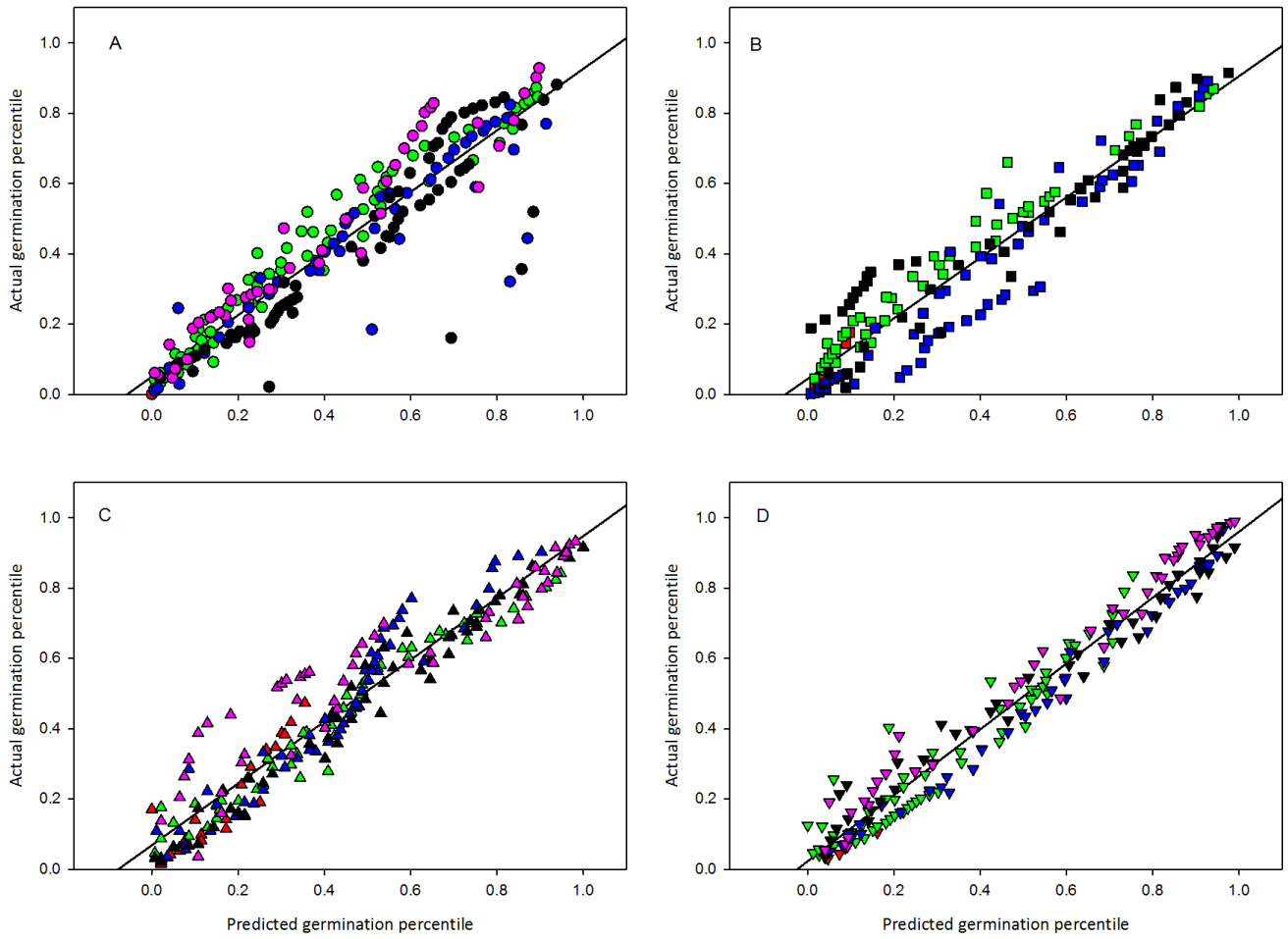


Figure 3-8 Predicted against actual germination percentile for sub-optimal temperatures for A) brome (●), B) cocksfoot (■), C) perennial ryegrass (▲) and D) tall fescue (▼) seeds at 0 MPa, under different temperatures (● = 5, ● = 10, ● = 15, ● = 20 and ● = 25 °C).

In supra-optimal temperatures, germination time by HTT model did not predict germination time well since predicted germination time was overestimated when T was 35 °C for brome, perennial ryegrass and tall fescue and was 30 °C for cocksfoot (Figure 3-9). Final germination percentage and GR for germinated seeds at the mentioned temperatures were higher than the actual values.

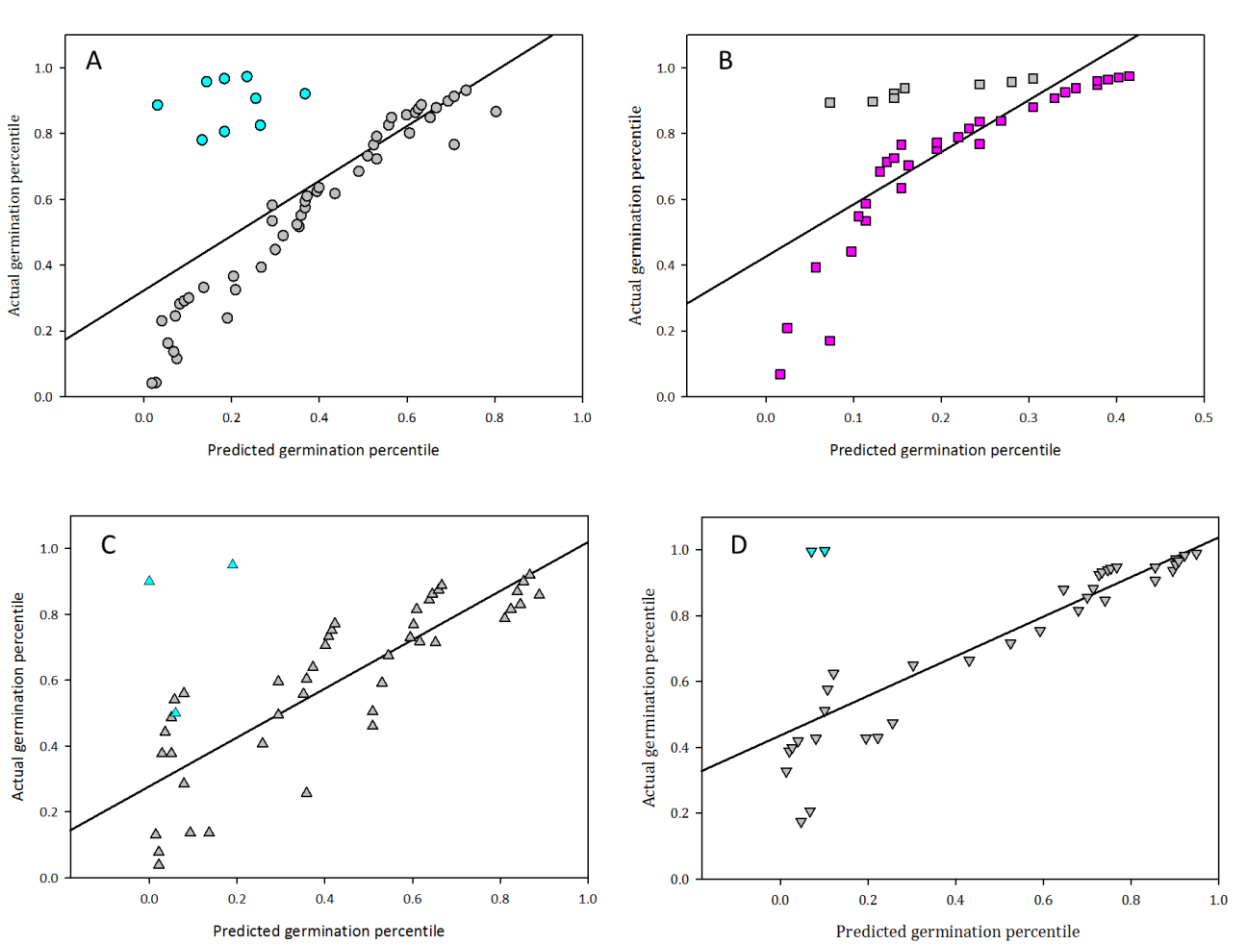


Figure 3-9 Predicted against actual germination percentile for supra-optimal temperatures for A) brome (●), B) cocksfoot (■), C) perennial ryegrass (▲) and D) tall fescue (▼) at 25 °C ▲, 30 °C ▲, and 35 °C▲.

When k value was applied for all temperature treatments at supra-optimal temperatures, the HTT model predicted germination time reasonably well for perennial ryegrass, but still did not work for brome, cocksfoot or tall fescue (Figure 3-10).

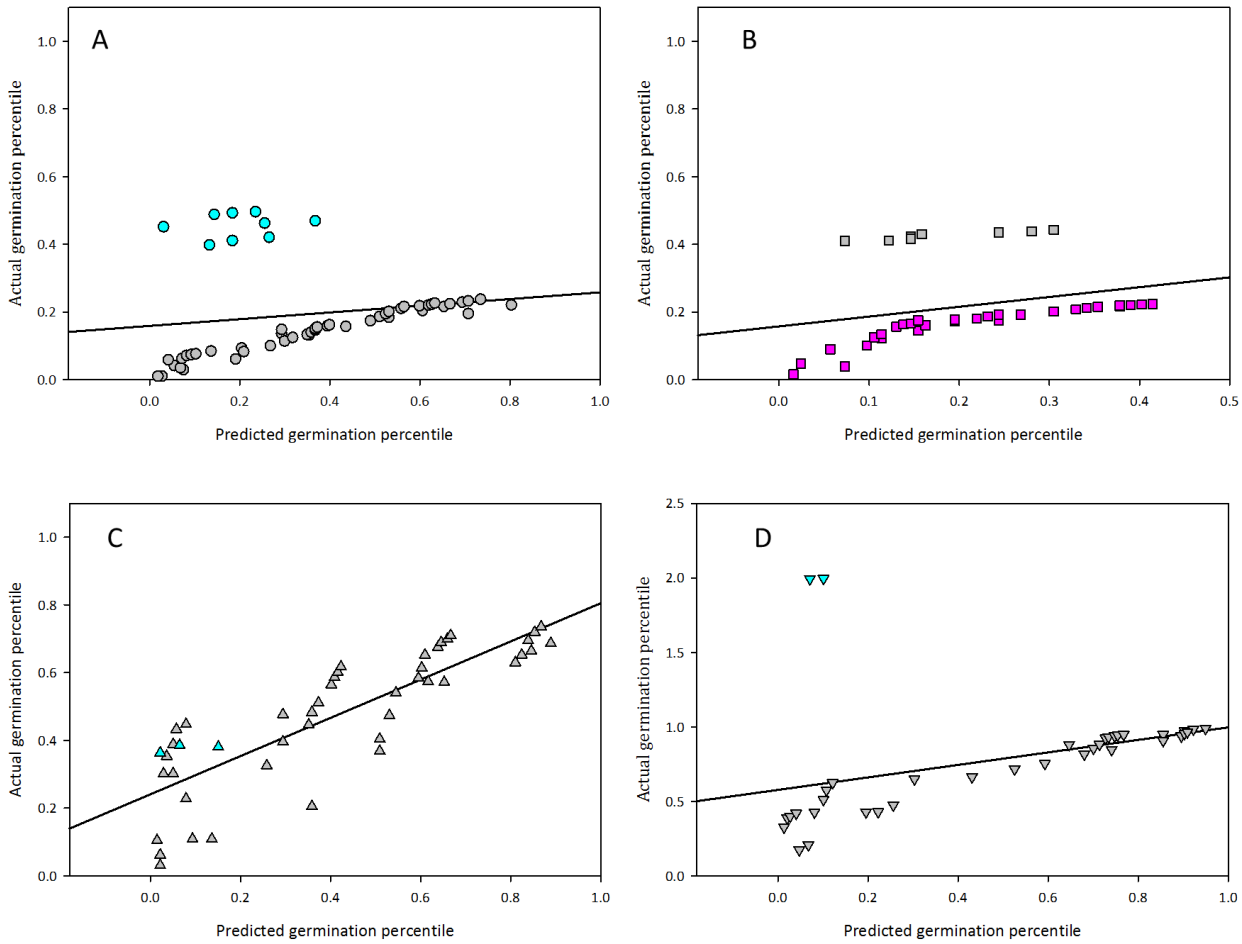


Figure 3-10 Predicted against actual germination percentile for supra-optimal temperatures (25 °C ▲, 30 °C ▲, and 35 °C▲), for A) brome (●), B) cocksfoot (■), C) perennial ryegrass (▲) and D) tall fescue (▼) when K value applied for all supra-optimal temperatures. The scales are not similar.

Applying K value only for 25 °C and 30 °C for cocksfoot, and 30 °C for brome, perennial ryegrass and tall fescue in the supra-optimal range of temperatures, meant germination time was predicted reasonably well for brome, cocksfoot, perennial ryegrass and tall fescue (Figure 3-10).

3.6 Discussion

3.6.1 Implications for pasture establishment

This study presented differences in germination responses of brome, cocksfoot, perennial ryegrass and tall fescue to WP and temperature treatments. These have implications for their germination under field conditions. Seeds need to accumulate a certain amount of thermal time for germination (Moot *et al*., 2000; Larsen *et al*., 2005; Lonati *et al*., 2009; Monks *et al*., 2009). However, under rainfed field conditions, important differences in germination behaviour appear to be driven by differences in their responses to soil moisture interacting with thermal time accumulation. This new information can help in sowing date recommendations, particularly under dry conditions.

The current results showed that, 'Sfr36-009' cocksfoot germination rate and final germination% was relatively lower than 'Stellar AR1' perennial ryegrass, 'Finesse Q' tall fescue and 'Bareno (9045D)' brome, specifically when higher temperatures (T≥25 °C) were combined with lower moisture (≤-0.37 MPa). This may cause a failure in the establishment of rainfed pastures in the autumn when the average soil temperature is normally between 25 °C and 35 °C, and also soil moisture can be below 15-18%. It took almost 10 days for more than 50% of cocksfoot seeds to germinate under colder soil temperature (10≤T≤20 °C) when WP was 0≥WP≥-0.37 MPa which is equal to ~15% soil moisture. This is similar to the rainfed spring sown pastures when the average soil temperature is less than 25 °C or rainfed autumn sown pastures once a light autumn rainfall has decreased soil temperature and increased soil moisture. However, increasing temperature to 25 °C, decreased both germination rate and the final germination% for cocksfoot, which may cause a failure in its establishment at the same level of moisture stress (-0.37 MPa). Based on these results, under this condition sowing cocksfoot is not recommended. Previous studies have shown that cocksfoot is able to compete effectively with weeds and other pasture plants for resources once established (Borman *et al*., 1990). However, compared with perennial ryegrass, it is relatively slow to establish (Moot *et al*., 2000; Turner *et al*., 2006), which continues to hinder farmer acceptance of its use in the dairy industry (Turner *et al*., 2006).

These results suggest that for rainfed pastures, more than 90% of the sown 'Finesse Q' tall fescue seeds would germinate, once the average soil temperature is 15-25 °C and soil moisture content is more than 15% for a silt loam soil and 18% for a clay soil (-0.37 MPa) (Sharifiamina *et al*., 2016). In dryland autumn sown pastures the average soil temperature can be 30 °C or higher. At 30 °C, more than 70% of tall fescue seeds can be expected to germinate under -0.18 and -0.37 MPa moisture stress which is almost equal to 15-18% for a silt loam soil type and 18-20% for a clay soil type. Under the same temperature range, more than 70% perennial ryegrass and brome seeds germinated even at the lower WP (-0.63 MPa) which is equal to 14 and 16% soil moisture, for a silt loam and clay soil type, respectively. Final

germination% of ‘Sfr36-009’ cocksfoot was relatively lower than ‘Stellar AR1’ perennial ryegrass, ‘Finesse Q’ tall fescue and ‘Bareno (9045D)’ brome. Once the average soil temperature was 30 °C, and water was not limited (0 MPa), almost 50% ‘Sfr36-009’ cocksfoot would germinate. Decreasing soil moisture to 17 and 20% for a silt loam and clay soil types respectively results in 30% germination at 30 °C. Once moisture decreased below that at 30 °C, typical of dry soils, it caused a rapid decline in germination. In this situation, sowing cocksfoot seeds is not recommended particularly in light sandy soils. Moisture stress beyond -0.37 MPa at 30 °C caused a rapid decline in germination of tall fescue and moisture stress beyond -0.63 MPa at 30 °C, caused a rapid decline in the final germination% of brome and perennial ryegrass. These levels of moisture deficit are common in dry soils in the autumn. In this situation, sowing tall fescue seeds is not recommended particularly in light sandy soils. Since 50% of cocksfoot seeds are able to germinate at 30 °C only when water was not limited, and they did not germinate at 35 °C even when water was not limited, sowing cocksfoot under these conditions is not recommended.

3.6.2 Differences in seed germination among cultivars

The current study shows different responses of the four species to WP and temperature which has implications for their germination and establishment under field conditions.

When moisture was non-limited (WP = 0 MPa), among all the species in this study, the optimum range of temperatures for brome was the highest and for cocksfoot was lowest. This shows that, if autumn sown, when soil temperature ≥25 °C, ‘Bareno’ brome should be able to germinate almost two days earlier than ‘Sfr36-009’ cocksfoot. Comparing the results of the current study with previous studies showed that improvements in seed germination rate have resulted from breeding and selection within the cocksfoot genus. Germination of newly bred ‘Sfr36-009’ cocksfoot used in this study was similar to newly bred ‘Tekapo’ cocksfoot and faster than ‘Kara’ cocksfoot reported by Turner *et al*. (2007). Germination of ‘Bareno’ brome was similar to the same brome cultivar (Turner *et al*., 2007). Faster establishment is originally accepted as an attribute of ‘Kara’ cocksfoot compared with the other cocksfoot cultivars. However, in their glasshouse experiment, when moisture was not limited, it took 5.9 and 8 days for ‘Tekapo’ and ‘Kara’ cocksfoot respectively to start emergence when glasshouse conditions were controlled to maintain day/night temperatures of 20/10 °C.

3.6.3 Observed and predicted germination

Germination behaviour for tall fescue was accurately described by the HTT model in the sub-optimal range of temperatures (Figure 3-8 D). However, in some cases, there were some biases under certain temperature/WP treatments for perennial ryegrass, cocksfoot and brome. For perennial ryegrass, germination percentile was overestimated by the model in sub-optimal range of temperatures, only

when WP=-0.95 MPa at 25 °C (Figure 3-8 C). At the sub-optimal range of temperatures for cocksfoot, germination was underestimated by the model at 15 °C (Figure 3-8 B), when WP was -0.63 MPa. Predicted germination for cocksfoot by the model was close to actual germination percentile when WP was 0 and -0.18 MPa. However, when WP was decreased to -0.37 MPa, the model over predicts germination at 20 °C (Figure 3-4 B). When WP was -0.18 MPa for brome, predicted germination time (Figure 3-4 A) and germination percentile at sub-optimal temperatures, was less close to the actual germination time which was due to underestimating germination time and the final number of germinated seeds (Figure 3-9). Although seed imbibition is as a result of differences in the seed and the ambient WP, decreasing WP to -0.18 MPa for brome seeds did not change germination rate and the final number of germinated seeds when 15≤T≤30 °C. This might be due to two possible reasons:

1) Decreasing WP by using PEG might not have affected the germination of brome seeds. As it was explained in Section 3.3, in some previous studies also, germination rate and the final germination of some species with different seed shape and size were increased when they were directly in contact with PEG solutions. In the current study also, compared with cocksfoot, tall fescue and perennial ryegrass seeds, brome seed size and shape was different. Brome seeds are larger (~7.5×0.2 mm) than other grass seeds used in this study. This means that the area which is in contact with the PEG solution is relatively larger than other grass seeds.

2) There might be some physiological mechanisms promoting tolerance of moisture stress which enabled the brome seeds to germinate under mild moisture stress of -0.18 MPa. This possibly was not observed in the other species in the current study.

Overestimating germination percentile by the model, also happened at the highest temperature at the range of sub-optimal temperatures when WP was less than -0.37 MPa for perennial ryegrass and brome (at 25 °C), and when WP was less than -0.18 MPa for cocksfoot (at 20 °C). A possible reason is that the HTT model predicts thermoinhibition only as a function of temperature at supra-optimal range (k[T-To]), but thermo-inhibition of all species in this study appeared to be sensitive to decreasing WP (less than -0.37 MPa) in temperatures below To, as well as increasing temperature above To. This possibility needs further research. In other words, as WP decreased below -0.37 MPa for perennial ryegrass and brome (at 25 °C), and -0.18 MPa for cocksfoot (at 20 °C), the optimum temperatures and as a consequence sub-optimal range was decreased. At supra-optimal temperatures, when temperature was 35 °C for brome, perennial ryegrass and tall fescue and 30 °C for cocksfoot, germination time and final germination percentile predicted by HTT model was overestimated under all WP treatments. Using the “k” value, did not increase the accuracy of the model for two reasons. First, increasing temperature by 5 °C at the supra-optimal range caused a rapid decline in final germination% and GR for all species. This was particularly under decreased WPs and in many cases, no

seeds germinated under the combination of supra-optimal temperatures and decreased WP treatments. Therefore, there was limited data at supra-optimal temperatures to fit the linear regressions to Ψb (50)s versus temperature to calculate an accurate "k" value. The second reason is that, to calculate a "k" value based on the HTT model, a single optimum temperature should be considered for each species. The optimum temperature is defined as the temperature in which GR is maximum under non-limiting moisture. However, the results of the current study suggest that, the optimum temperature might be different for different WPs. As an example, for perennial ryegrass, once T was 30 °C, GR was maximum when WP was 0 MPa. However, by decreasing WP to -0.63 MPa at the same temperature, GR decreased rapidly, therefore GR was overestimated by the HTT model.

3.6.4 HTT model fitted

The hydrothermal time model has been frequently used to predict seed germination behaviour based on the interaction between the physiological responses to temperature and WPs (Bradford, 1995, Rowse and Finch-Savage, 2003). Using this model, seed germination times across the range of sub-optimal temperatures and WPs can be described with accuracy (e.g. Dahal and Bradford 1994). In the current study also, although germination behaviour of all grass species was predicted reasonably well (R^2≥91%) at sub-optimal temperatures, in certain cases, the predicted germination curve fitted poorly with the observed germination. There was an overestimation in predicted germination at the higher temperature treatments at sub-optimal temperatures when WP was decreased. As an example the HTT model overestimated germination time and the final germination% of cocksfoot when WP decreased to -0.63 MPa at 20 °C. This means that the assumption of having a normal distribution in Ψb (g) has been rejected in this case. Larsen *et al*. (2004), also reported an overestimation in the predicted germination curves when WP was –1.5 MPa at 25 °C in two red fescue cultivars. They assumed that the reason for this may possibly be that the actual WP in the germination medium (which was not measured frequently during the experiment), was different than the expected WP, probably due to evaporation of water during the experiment. However, if their argument is correct, and evaporation is the case, it would happened equally for all WP treatments and so, estimation of germination in WPs higher than -1.5 MPa (including -0.2, -0.4, -0.6, -0.8, -1 and -1.25 MPa) which were incubated at the same temperature (25 °C) should also be overestimated by the model at the same level. In the current study, this may not be the case since WPs of the solutions were checked every four days (Section 3.2). Therefore, it can be concluded that inability of the HTT model to predict germination in the supra- optimal temperatures, specifically under decreased WP can be the reason for over predicting germination rate and final germination percentage in their study as well as the current research. The specified model by Equations 2-12 and 2-13 applies at sub-optimal and optimum temperatures (Section 2.10).

Models of Alvarado and Bradford (2002) and Rowse and Finch-Savage (2003) assumed a normal distribution of Ψb (g) and a linear upward shift in Ψb (g) with increasing temperature at supra-optimal temperatures. As described in Section 3.4.1 the probit-based model described the thermoinhibition response as a function of raising the threshold WP for seed germination (seed base WP, Ψb (g)). These models assume a normal distribution of Ψb (g) and a linear upward shift in Ψb (g) with increasing temperature. However, the assumption that Ψb (g) is normally distributed within these models has not been interrogated (Watt *et al*., 2010). The current study examined whether the decline in germination percentage and germination rate (GR) in brome, cocksfoot, perennial ryegrass and tall fescue seeds observed at supra-optimal seedbed temperatures could be accurately predicted using a similar hydrothermal model to that proposed by Alvarado and Bradford (2002) and Rowse and Finch-Savage (2003), where Ψb (g) is adjusted upwards with increasingly supra-optimal temperatures. Based on the results of this research, increasing Ψb (g) of the species used in this study did not follow a normal distribution and there was not a linear upward shift in Ψb (g) with increasing temperature at supra- optimal temperatures. Results show that, at supra-optimal temperatures, thermo-inhibition of all species in this study appeared to be sensitive to decreasing WP (less than -0.37 MPa) at the range of optimum temperatures, as well as supra-optimal temperatures, and this possibility needs further research. This suggests that both at optimum and supra-optimal temperatures defined for each species, there might be an interaction between temperature and WP treatments caused an unexpected rapid decline in the final germination% and GR of the seeds, once WP decreased to ≤-0.37 MPa.

The results of the current study are consistent with the results reported by Watt *et al*. (2011) for *B*. *davidii* Franch. (buddleja), *P*. *radiata* D. Don (*radiata pine*), *Allium cepa* L. cv.Hyton (onion) and *Daucus carota* L. cv.*Narman* (carrot). Watt *et al*. (2011) showed that at supra-optimal temperatures, not considerable thermoinhibition happened for the earliest germinating seeds under 0 MPa. This means that the seeds germinated very rapidly under moist conditions at supra-optimal temperatures. However, for the rest of the population, decreasing WP below 0 MPa caused a progressive rise in Ψb (g) at supra-optimal temperatures so they have suggested that the slower germinating seeds were thermoinhibited.

3.7 Conclusions

- The maximum final germination percentage for cocksfoot was 20-40% when T=30 °C and moisture was not limited. Decreasing WP (increasing moisture stress) at 30 °C resulted in ≤20% germination. Therefore, under dryland conditions, sowing cocksfoot is not recommended when the average soil temperature is 30 °C or more.
- There was a wider range of optimum temperatures for perennial ryegrass (20-30 °C) compared with cocksfoot (20-25 °C). The highest thermal time requirement for 50% of the final germination (Tt50) was 114 °Cd for cocksfoot and the lowest was 90 °Cd for perennial ryegrass.
- The base temperatures were between 1.5 °C for perennial ryegrass and 4 °C for brome. Ceiling temperatures were between 35-40 °C for all species.
- Cocksfoot was relatively sensitive to increasing temperature and decreasing WP. More than 50% of cocksfoot seeds germinated only under a narrow range of 10 ≤T≤20 °C when 0≥WP≥-0.37 MPa.
- Except when temperature was 15 °C, under all temperature treatments, final germination percentage of cocksfoot was 20-40% when WP=-0.63 MPa. The optimum temperatures for cocksfoot were 20 and 25 °C when moisture was not limited. However, 15 °C is the optimum temperature in which 41-60% of cocksfoot seeds germinated when the level of moisture stress was 14 and 16% soil moisture for silt loam and clay soil types respectively (WP=-0.63 MPa).
- Tall fescue germination was accurately predicted by the HTT model.
- Prediction of GR and the final germination percentage using the hydrothermal time model was accurate at sub-optimal temperatures. However, there were some exceptions. The HTT model also overestimated GR and the final germination percentage under decreased WP at To.
- Both GR and final germination percentage of brome was unaffected by decreased WP at -0.18 MPa when temperature was 15 and 20 °C. This was probably due to the different seed shape and size for brome compared with the other species which resulted in increasing the hydraulic conductivity under a certain WP.

Chapter 4 Agronomic performance of brome, cocksfoot, perennial ryegrass and tall fescue monocultures

4.1 Introduction

Chapter 3 investigated the combined effects of moisture and temperature on seed germination of brome, cocksfoot, perennial ryegrass and tall fescue. The results were consistent with previous studies that highlighted perennial ryegrass is able to germinate under a wider range of soil moisture deficit and temperature compared with narrower optimum ranges for tall fescue, brome and cocksfoot.

Perennial ryegrass establishment is faster than tall fescue, brome and especially cocksfoot (Charlton and Stewart, 1999). However, severe summer moisture deficits restrict perennial ryegrass pasture production and persistence (Woodman *et al*., 1992). Previous studies have shown that when temperature was not limited in dry summer and autumn periods in Canterbury, dry matter production of dryland cocksfoot was higher than ryegrass (Stevens *et al*., 1992, Mills *et al*., 2006). In addition to cocksfoot, tall fescue (Charlton and Stewart, 2006, Ebrahimiyan *et al*., 2013, Turner *et al*., 2012) and brome (Laude, 1953) have been reported to be more productive than perennial ryegrass under summer dry conditions (Section 2.8.6).

Nitrogen deficiency as well as moisture stress can cause yield reductions (Section 2.1). Nitrogen is the most limiting nutrient and is essential for growth of all plants (Grindlay, 1997; Azam, 2002). Therefore, evaluating the combined effects of moisture and N on dry matter production and the quality of monocultures of rainfed brome, cocksfoot, perennial ryegrass and tall fescue is necessary to understand mechanisms responsible for yield responses.

This chapter deals with Objective 2 which is to generate pastures with contrasting yield potential through the two sites which differ in PAWC.

Objective 3 is to quantify the seasonal production and quality of these pastures during their establishment (2014/15) and first year (2015/16). Since variations in pasture establishment among different species might affect pasture production after establishment, the seedling number and root and shoot biomass of dryland brome, cocksfoot, perennial ryegrass and tall fescue were quantified at both sites in the establishment year (2014/15). Total N recovery of the pastures was compared among ±N species to investigate if under dryland conditions there is any interaction between species and N at each site.

The analysis also deals with Objective 4 to quantify DM production in relation to accumulated thermal time. The relation between accumulated thermal time and DM production available in the literature (Section 2.2.2) mostly accounted for periods when neither moisture nor N were limited. However, in dryland pastures, the main constraints, other than temperature, affecting pasture production are moisture stress and N availability. The current study will describe the relationships between accumulated thermal time and accumulated DM production of monocultures under different levels of moisture and N availability. This will allow a more accurate description of DM production typical of New Zealand dryland pastoral systems.

4.2 Materials and Methods

In this chapter the methodology and data analysis protocols for two field experiments are described. Then the results are presented in this chapter, Chapters 4, 5 and 6.

4.3 Experimental sites

The same experimental design and treatments were established at two sites. Site 1 is at Ladbrooks (43.37'S, 172°.30'E, 12 m.a.s.l) where Experiment 1 was established on 0.357 ha of flat land in 2014. It is a Field Research Farm for Seed Force Limited and was monitored through the 2014/15 and 2015/16 seasons.

Experiment 2 was established at Ashley Dene, which is located 14.5 km south west of Site 1. Experiment 2 used 0.275 ha and is located in a flat land in Paddock C8 of Ashley Dene (43°39'S, 172°20'E, 30 m a.s.l), the Lincoln University Dryland Research Farm.

4.4 Paddock history

4.4.1 Experiment 1 at Ladbrooks

In 2012 this site was planted in kale (*Brassica oleracea* L.) and rape (*Brassica napus* L.). In March 2013 annual ryegrass (*L. multflorum* L.) was sown and died out in January of 2014. When annual ryegrass was sown in March 2013, a mixture of Urea and 200 kg ha^{-1} yr^{-1} of Cropmaster 20 (NPKS: 19.3, 10, 12.5, 0) was applied to the entire area. From January to September 2014, immediately prior to this experiment, the area was uncultivated. It was sprayed with glyphosate at 1.4 kg a.i ha^{-1}, at the end of September 2014.

4.4.2 Experiment 2 at Ashley Dene

Paddock C8 at Ashley Dene had been uncultivated for more than 60 years. The resident vegetation included: brown-top, white clover, twitch (*Elytrigia repens* L.), and cocksfoot.

4.5 Soil characteristics

4.5.1 Experiment 1 at Ladbrooks

Ladbrooks site is a flat to very gently undulating land with severe drainage/permeability restrictions. The experimental site is located over the boundary of an imperfectly drained Wakanui silt loam (*Mottled Immature Pallic* or *Aquic Haplustepts*, USDA Soil Taxonomy) soil at the northeast end and a poorly drained Temuka clay (*Typic Orthic Gley*, USDA Soil Taxonomy) at the southwest (http://smap.landcareresearch.co.nz). Ladbrooks soil is deep (>1 m) and stone-less with 50-100 cm potential for rooting depth (http://smap.landcareresearch.co.nz). It has very poor drainage and very limited aeration in the root zone. Water logging vulnerability at Ladbrooks site is high and the drought vulnerability (if irrigated) is low.

4.5.2 Experiment 2 at Ashley Dene

Paddock C8 is a shallow soil and a strong pan exists with a variety of thickness at different soil depths. The available soil moisture in gravel layers is low and rooting is usually less than 700 mm of soil depth. Paddock C8 is on a Lismore stony silt loam (*Typic Dystrustept*, USDA Soil Taxonomy) area on its west side and a deep fine sandy loam area on its east side. The deep fine sandy loam soil is mainly stony with some soil channels up to 400 mm of stone-free materials. It is moderately well drained and the total available soil water content was reported as 100-160 mm. The Lismore stony silt loam area has excessive drainage and 450-750 mm of the stone depth overlaying horizons of coarse gravels in firmly packed sandy loam (http://smap.landcareresearch.co.nz).

4.6 Meteorological conditions

4.6.1 Measurements

Precipitation, soil and air temperature were measured at each site. A thermistor was set up at 1.5 m above the ground to measure air temperature. Air temperature was measured on an hourly basis by a 'Hobo 4-channel logger' (Onset Computer Corporation, Bourne, Maryland, USA) installed at the middle of each experimental site. Soil temperature was recorded hourly over the two years from sowing date at the depths of 10, 15, and 20 mm. This is reported from sowing date to 29/2/2015, at the depth of 10 mm at Ladbrooks (Appendix B) and Ashley Dene (Appendix C).

Windrun (km d^{-1}), solar radiation ($MJ/m^2/d$), wet and dry bulb temperatures were recorded at Lincoln Broadfields Meteorological Station (NIWA, National Institute of Water and Atmosphere Research, New Zealand) located 3.3 km west of Ladbrooks and 12 km north east of Ashley Dene. Measurements were logged hourly and daily means were calculated.

4.6.2 Long-term mean meteorological data (LTM)

Long term mean for meteorological data are from 1975-2012 and are presented in Table 4-1. Canterbury's climate is categorised as cool and temperate with an average annual rainfall of 637 mm equally spread over the year. Annual mean temperature is 12 °C, ranging from an average of 6.3 °C in July to 17.1 °C in January (Table 4-1). Annual average Penman (EP) is 76.7 mm which normally surpasses precipitation from September to April.

Table 4-1 Monthly means from 1975 to 2012 for total solar radiation (Ro), maximum (Tmax), minimum (Tmin) and mean (Tmean) air temperatures (°C), rainfall (mm), Penman potential evapotranspiration (EP), wind run (km d^{-1}), and vapour pressure deficit (VPD). From 1975-2000 measurements were taken at EDL Broadfields Meteorological Station (Open paddock, 2 km northwest of Lincoln township), Canterbury, New Zealand. From 2000 to 2012, measurements were taken from EWS Broadfields Meteorological Station (Open paddock, 200 m northeast of EDL site), Lincoln, Canterbury, New Zealand.

Month	Ro	Tmax	Tmin	Tmean	Rainfall	EP	Windrun	VPD
	$MJ/m^2/d$	(°C)	(°C)	(°C)	(mm)	(mm)	km d^{-1}	(KPa)
January	22.7	21.1	11.6	17.1	45.2	140	398	1.4
February	19.5	22	11.9	17	43	112	387	1.4
March	14.9	20.6	9.6	15.1	51.3	89.3	359	1.3
April	9.4	17.2	6.7	12	48.2	46.3	318	1.1
May	6.1	14.4	4.3	9.4	51.5	27.1	296	0.9
June	4.6	11.8	2	6.9	61.6	16.4	271	0.8
July	5.4	11	1.5	6.3	69	18.4	274	0.7
August	8	12.5	3	7.9	63	34.5	321	0.8
September	12.7	14.8	4.8	9.9	42	59.7	353	1.0
October	17.7	17.3	6.6	12.1	50.5	97.8	385	1.1
November	22.3	18.7	8.3	13.6	54.4	126	391	1.2
December	23.1	21	10.4	15.7	54.1	144	389	1.3
Annual	13.9	16.9	6.7	11.9	637	76.7	345	1.1

4.6.3 Rainfall and evapotranspiration during the experiments

From October to December 2014 in the first year of the experiment, monthly rainfall was 5-25 mm less than a long term mean at the both sites (Figure 4-1 A). Total rainfall in 2015 was 325 mm compared with the 637 mm of long term mean. Seasonal distribution did not follow the long term patterns. Except for April, June and September of 2015 in which accumulated rainfall was 10-25 mm more than the long term average, in other months total rainfall was 10-30 mm less than the average (Figure 4-1

A). Rainfall in January and May 2016 was ~40 mm and 90 mm more than long term mean at Ashley Dene. In the other periods of the year, monthly accumulated rainfall was 20-30 mm less than the mean. At the same time, at Ladbrooks, monthly accumulated rainfall was between 40-60 mm less than the long term mean (Figure 4-1 A).Monthly PET followed the long term trend with a minimum monthly PET of 4-21 mm in August/July and a maximum of 144-158 mm in December/January. Annual PET in 2016 was 935 mm and 954 mm at Ladbrooks and Ashley Dene respectively (Figure 4-1 B).

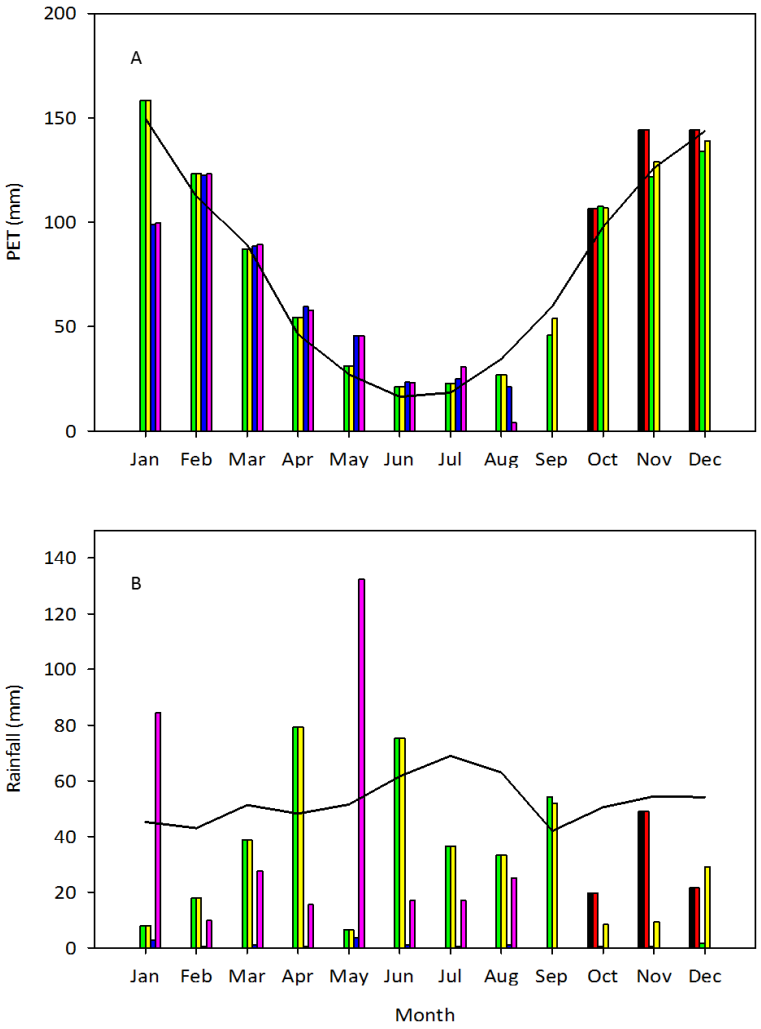


Figure 4-1 Meteorological data for the experimental period (October 2014 to August 2016). Data are shown (A) potential evapotranspiration (PET) and (B) monthly rainfall at Ladbrooks and Ashley Dene in 2014 (■,■), 2015 (■,■) and 2016 (■,■). Solid lines (—) are long-term (1975-2012) data from the Broadfields meteorological station located 3.3 km west of Ladbrooks and 12 km northeast of the Ashley Dene site.

4.6.4 Temperature and solar radiation

Total solar radiation and the average monthly air temperature followed the long term trend. The highest monthly temperature at the sites was in January 2016 (18.5 °C), and the lowest was 5.5 °C and

3 °C in August 2016 at Ladbrooks and Ashley Dene, respectively (Figure 4-2 A).The average monthly solar radiation was highest (23-25 MJ/m^2) in November/December of 2014 and 2015 and lowest (5-6.4 MJ/m^2) in June 2014 (Figure 4-2 B).

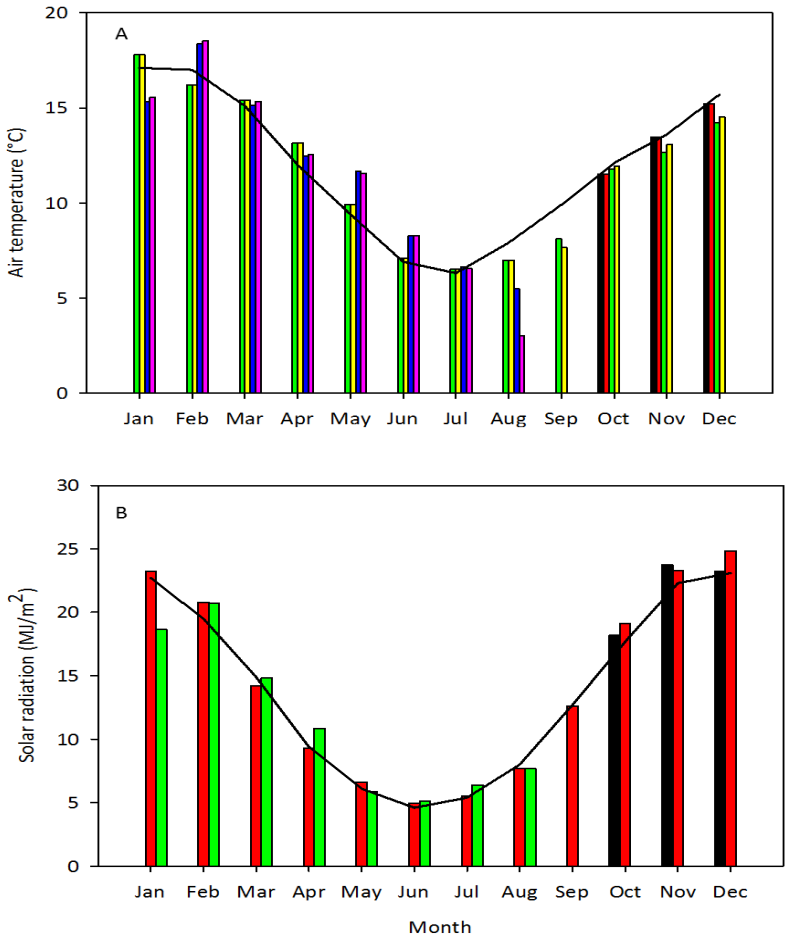


Figure 4-2 Meteorological data for the experiment period (October 2014 to August 2016). Data are shown (A) monthly air temperature at Ladbrooks and Ashley Dene in 2014 (■,■), 2015 (■,■) and 2016 (■,■) and (B) total solar radiation data in 2014 (■), 2015 (■) and 2016 (■). Solid lines (—) are long-term (1975-2012) data taken from the Broadfields meteorological station located 3.3 km west of Ladbrooks and 12 km northeast of the Ashley Dene site.

4.6.5 Vapour pressure deficit (VPD) and Windrun

The maximum average monthly VPD was 0.13 kPa in January 2016 and 0.15 in February 2016 and the minimum was 0.07 kPa in August 2016 (Figure 4-3 A). Wind run was highest in December 2014 (450 km) and was 260 km in August 2014 (Figure 4-3 B).

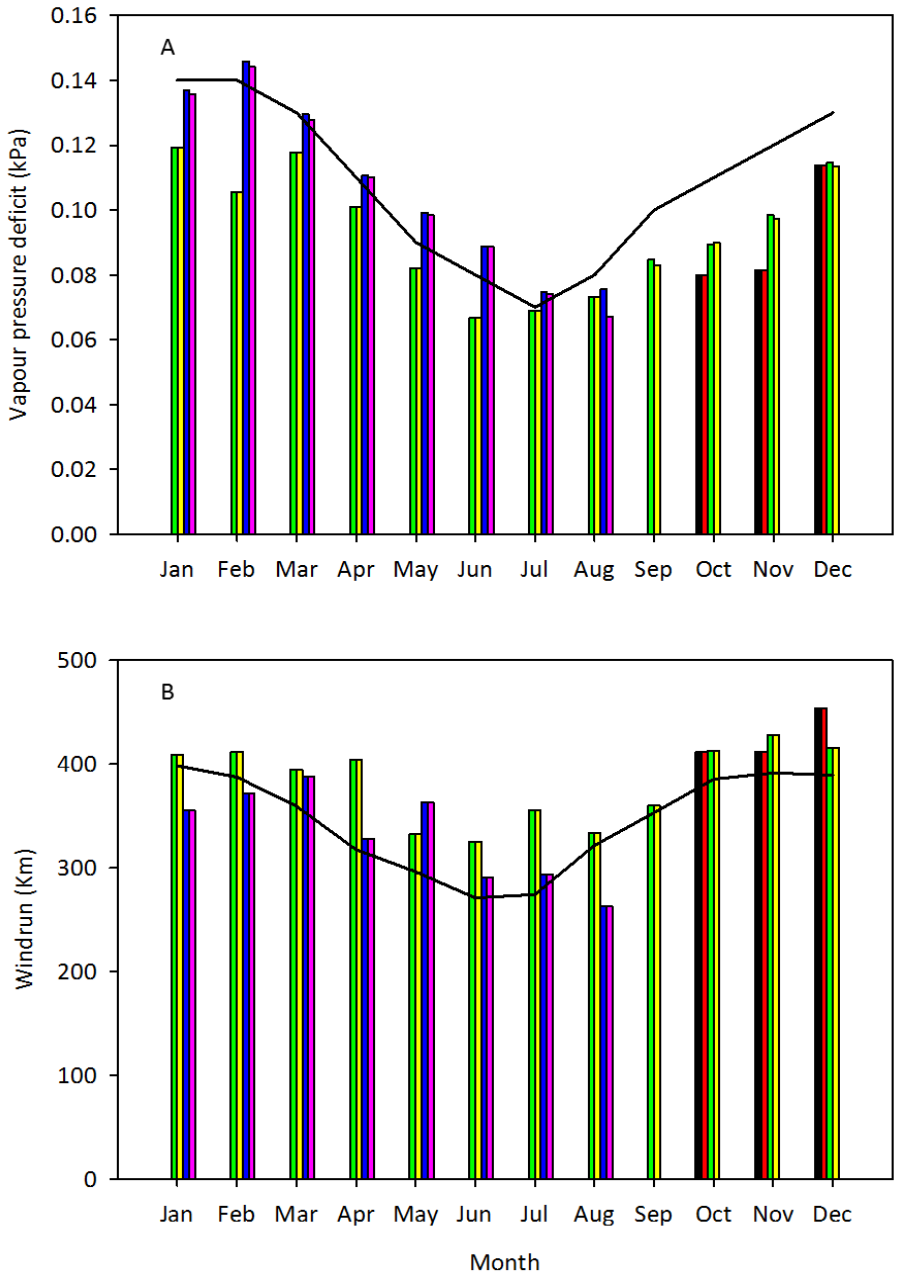


Figure 4-3 Meteorological data for the experiment period (October 2014 to August 2016). Data are shown monthly (A) vapour pressure deficit and (B) windrun at Ladbrooks and Ashley Dene in 2014 (■,■), 2015 (■,■) and 2016 (■,■). Solid lines (—) are long-term (1975-2012) data taken from the Broadfields meteorological station located 3.3 km west of Ladbrooks and 12 km northeast of the Ashley Dene site.

4.7 Agronomic management

4.7.1 Experimental design and treatments

In the first year of the study (2014/15), both Experiments 1 and 2 had a Latin square design with four grasses and four replicates. This was due to the environmental gradients including a ditch in the

Northern corner of the paddock and variability in background soil N (Section 4.7.4) which was extended from the North to South of the paddock at Ladbrooks. In the second year (2015/16), once background N was utilised by the grasses, the experimental design was modified to include ±N fertiliser treatments. The 16 plots at each site were halved to accommodate the additional treatments and the experiment was subsequently analysed as a strip-plot design with ±N as the rows and pasture species as columns (Appendix C). Nitrogen was applied as strip lines in each block and randomized within each block. There were 32 plots and individual plot sizes for the strip-plot experiment were 6.3 x 9 m for Experiment 1 at Ladbrooks and 6.3 x 10 m for Experiment 2 at Ashley Dene (Plate 4-1). For Experiment 2 at Ashley Dene, there were 3 m boundary lines between all the blocks. At Ladbrooks, the available area was limited, therefore there was no boundary line between the blocks.

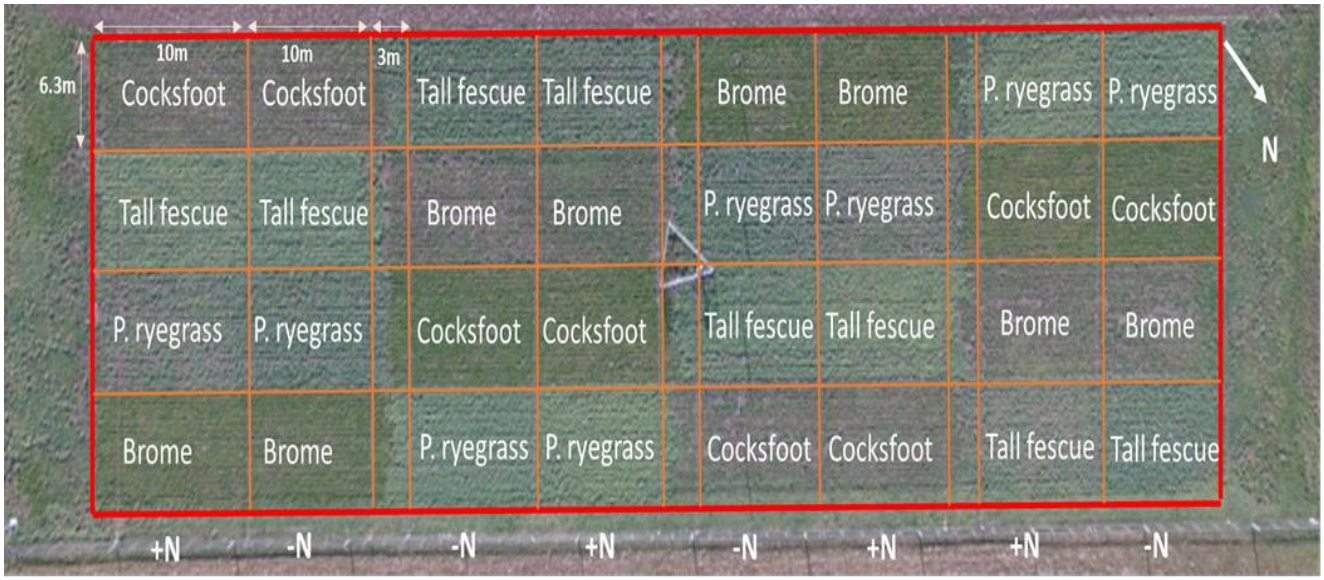


Plate 4-1 Ashley Dene experimental site on 10/7/2016. The single vertical orange lines separate strip-plots (rows), the single horizontal orange lines separate species treatments (columns), and double lines separate blocks. The strips in which nitrogen fertiliser was applied are shown by +N and those in which nitrogen fertiliser was not applied (control) are shown by −N.

Although in the first year of the study (2014/15) N fertiliser was not applied, the figures present the results separated into + and −N treatments both in 2014/15 and 2015/16 for completeness.

4.7.2 Seedbed preparation

Experiment 1 at Ladbrooks

MCPB (4-(4-chloro-o-tolyloxy) butyric acid; 1.5 kg a.i. ha^{-1}) was used to control weeds on 4/8/2014. The main weeds at Ladbrooks were fathen (*Chenopodium album* L.), wireweed (*polygonum arenastrum* L.), stinging nettle (*Urtica dioica* L.), shepherd's-purse (*Capsella bursa-pastoris* L.) and white clover (*T. repens* L.).

Experiment 2 at Ashley Dene

On 23/9/2014, Buster (glufosinate-ammonium; 1 kg a.i. ha^{-1}) at 5 L ha^{-1} was applied to control the weeds (fathen, twitch, browntop (*Agrostis capillaris* L.), wire weed, white clover and cocksfoot). On 1/10/2014 plant residuals were cut and carried off site and the experimental area was then ploughed.

4.7.3 Establishment

Experiment 1 at Ladbrooks

A single application of 350 kg ha^{-1} of Cropzeal 20 (NPKS: 8, 15, 13, 0·8) was applied to the site on 9/9/2014. On 10/10/2014 cultivation took place at Ladbrooks. On 16/10/2014 the site was Cambridge-rolled just before sowing.

Experiment 2 at Ashley dene

Experimental plots at Ashley Dene were ploughed, heavy rolled and then Dutch harrowed on 2/10/2014. On 13/10/2014, the area was rotary-hoed to produce a fine, consolidated seedbed. Then plots were Dutch-harrowed and Cambridge-rolled followed by a heavy roll on 14/10/2014. Plots were drilled on 15 /10/2014.

All seeds for Experiments 1 and 2 were newly harvested and provided by Seed Force Ltd. Maximum germination percentage, cultivars and sowing rate (kg ha^{-1}) of all seeds are shown in Table 4-2.

Table 4-2 Cultivars, sowing rate (kg ha^{-1}) and germination percentage of four grass species at Ladbrooks and Ashley Dene.

Species	Cultivars	Sowing rate (kg ha^{-1})	Germination percentage
P. ryegrass	Stellar AR1	20	96%
Cocksfoot	Sfr36-009	10	88%
Tall fescue	Finesse Q	25	95%
Brome	Bareno (9045D)	35	98%

4.7.4 Soil fertility

Soil samples were taken on 30/7/2014 at Ladbrooks (Experiment 1) and on 12/12/2014 at Ashley Dene (Experiment 2). The samples contained 12 cores taken randomly to a depth of 150 mm of the top soil from each experimental site (Table 4-3). Test results indicated that no additional fertiliser needed to be added to the soil in the first year (2014/15).

To assess the amount of N fertiliser required to be applied to the +N plots, on 9/9/2015 soil samples were taken from both sites.

In 2015, once plants were established at Ladbrooks, uneven growth was visible within different plots due to a high background N. This was either due to the residuals from previous crops in the site or N fertiliser applied to previous swards (Section 4.4.1). To balance the amount of N in those parts of the paddock, separate soil samples were taken from areas with an apparently high background N as well as other areas. Based on soil test results (Table 4-3), in the first application of N fertiliser, less N fertiliser was applied (Appendix F) to the area with the high N background (Table 4-3).

Table 4-3 Soil test results (0-150 mm) from Experiment 1 (Exp 1) at Ladbrooks and Experiment 2 (Exp 2) at Ashley Dene, Canterbury, New Zealand.

Year	Experiment / Site	pH	Olsen P ($\mu g\ ml^{-1}$)	SO4-S ($\mu g\ g^{-1}$)	Ca^{2+}	K^+ -------	Mg^{2+} (meq/100g)	Na^+ - -------	Available N ($kg\ ha^{-1}$)
2014	Exp 1/ Ladbrooks	6.5	83	25	18.4	0.51	2.34	0.39	----
2015	Exp 1/ Ladbrooks (a)	6.1	47	-	13.8	0.32	2.23	0.41	103
2015	Exp 1/ Ladbrooks (b)	5.9	73	-	18.5	0.53	2.72	0.48	128
2014	Exp 2/ Ashley Dene	5.4	19	-	6.4	0.23	0.84	0.14	---
2015	Exp 2/ Ashley Dene	5.5	12	-	6.3	0.17	0.75	0.15	88

Note: Background N from fertiliser application to the previous *L. multifolum* L. crop at Ladbrooks meant in 2015 samples were taken from (a) unaffected and (b) affected areas. The first N fertiliser application in 2015 to the +N treatments at Ladbrooks was applied to plots with higher levels of background and to account for observed differences.

Table 4-3 shows that levels of Ca^{2+} and Mg^{2+} at Ladbrooks were almost three times more than Ashley Dene in 2014 (18.4 at Ladbrooks compared with 6.4 meq/100 g at Ashley Dene). At the same time, Mg^{2+} and Na^+ were almost double at Ladbrooks compared with Ashley Dene.

4.7.5 Weed control after establishment

Experiment 1 at Ladbrooks

On 1/1/2015 the site was sprayed with Trimec (600 g L^{-1} mecoprop, 150 g L^{-1} MCPA and 18.7 g L^{-1} dicamba; 13.30 kg a.i ha^{-1}) to control fathen, wireweed, stinging nettle and shepherd's purse.

Experiment 2 at Ashley dene

In the summer of 2014/15, the Experiment 2 area was weedy. However, since there was only 40 mm of rainfall between 3/3/2015 to 27/4/2015, the pasture was under severe moisture stress. Under these conditions, application of herbicide could have damaged the sown pasture grasses and was not recommended. On 3/3/2015 the plots were hand weeded in an area of 1 m^2 around the neutron probe

tubes and a mower was used on 5/3/2015 to cut the weeds in the remaining areas of the plots between the tubes. This was done to ensure accurate measurements of grass water extraction.

After almost 80 mm of rainfall, on 4/5/2015, 3 L ha^{-1} Trimec (600 g L^{-1} mecoprop, 150 g L^{-1} MCPA and 18.7 g L^{-1} dicamba; 13.30 kg a.i ha^{-1}) were used to control the broadleaf weeds across Experiment 2.

4.7.6 Mowing and grazing

Experiment 1 at Ladbrooks

At the end of each growth cycle, herbage was mown to a residual cutting height of ~30 mm with a Field Master Forage Harvester with a collection cage. This machine was unable to mow around and between the neutron probe access tubes. So, an area of 2 m^2 around each tube was hand clipped followed by using a lawn mower to cut the grasses in the area between the tubes. On all mowing dates, mown herbage mass was carried with the collection cage and removed from the experimental site. The frequency of mowing and regrowth periods for Experiment 1 are given in Appendix E.

Experiment 2 at Ashley Dene

Due to the stony soil at Ashley Dene, mowing as at Ladbrooks and using machinery was impossible. So, at this site, ewes grazed the pasture at the end of each growing period. Ewes were removed when residual grass height was ~30 mm to prevent overgrazing. The grazing dates, number of ewes, and the duration of grazing are given in Appendix E.

4.7.7 Nitrogen fertiliser

Experiment 1 at Ladbrooks

In 2015/16, a total annual application of 900 kg N ha^{-1} yr^{-1} was applied in seven split applications of 100 kg N ha^{-1} and one application of 200 kg N ha^{-1}, at the beginning of each regrowth cycle.

On 18/9/2015, only 50 kg N ha^{-1} was applied to the 6 +N plots (plots 14, 15, 18, 19, 22 and 23) located in the areas with background N (Table 4-3). 100 kg N ha^{-1} was applied to the rest of +N plots on the same date.

Experiment 2 at Ashley Dene

A total of 500 kg N ha^{-1} yr^{-1} was applied at Ashley Dene in 2015/16 in five split applications of 100 kg N ha^{-1}. Nitrogen fertiliser was applied before forecasted rainfall in each application time. The details of N fertiliser application at the both sites are available in Appendix F.

4.8 Measurements

4.8.1 Seedling's shoot and root biomass

On 16/12/2014, shoot and root biomass of seedlings were assessed by measuring 1 m of drill rows from each plot. The grasses were excavated from the soil using a spade. The digging process ensured minimal damage to adjacent rows. Sample plants were then transferred to the laboratory where soil was washed gently from roots to prevent the loss of fine roots. After air-drying, root and shoot were separated from the crown and transferred separately to a forced draft oven set at 60 °C until samples reached a constant weight.

4.8.2 Dry matter production

Destructive measurements

The management strategies employed in this research aimed at minimising seedhead production and dead material. Therefore cutting intervals were flexible based on the weather conditions. Regrowth periods of 35 to 50 days occurred during active growth (from August to March) and these extended to a maximum of 70 days over winter (from June to August) and during drought periods (April and May).

At Ashley Dene, regrowth periods were 80-90 days from July to March and extended to a maximum of ~150 days over winter (from June to August) or during drought periods (from December to May).

Samples for dry matter measurements were taken from a 0.2 m^2 quadrat with a set of electric shears to a residual pasture height of ~30 mm. The area harvested at the previous sampling was excluded. After cutting, sample bags were taken to the laboratory and left in an oven (60 °C) for at least 48 hours and the dry weight of each sample was measured.

Non-destructive

Non-destructive pasture production measurements were taken every 7-14 days (more frequently during active growth) during each regrowth period from 30/9/2015 to 30/7/2016. To do that, a folding pasture plate meter (Filips Ltd., Feilding, New Zealand) was used. To calibrate the pasture plate meter, in addition to the destructive harvests at the end of each regrowth period, some destructive harvests were made from the tallest (urine patches and/or in +N plots) and shortest plants (low nitrogen patches and/or in −N plots) in all plots within each regrowth period. A linear regression between pasture plate data and harvested plants allowed non-destructive estimation of dry matter production to be estimated through paired comparisons (Vickery *et al*., 1980).

Also, to obtain the average residual herbage height at each site, a pasture rising plate was used immediately after each mowing (Ladbrooks) or grazing (Ashley Dene) time.

4.8.3 Botanical composition

For each destructive harvest, botanical composition was measured from the sub-samples which were taken randomly from each plot using a quartering technique (Cayley and Bird, 1996). The harvested materials were transferred to the laboratory and sorted into sown grass, weeds and senesced herbage. If leaves were more than 50% dead, they were included in the senesced category. The harvested plant bulk (including weeds and the main grass treatments) was then dried in a forced draft oven at 60 °C until reaching a constant weight.

4.8.4 Nutritional and foliar analysis

Near infrared spectroscopy (NIR) was used to define metabolisable energy (ME) and the percentage of N. To do this, dried green samples were ground in a mill to pass through a 1 mm stainless steel sieve (Cyclotec Mill, USA). Nutritional analysis was not done on any other material (dead or weeds). Equation 4-1 (Kyriazakis and Oldham, 1993) was used to calculate crude protein and Equation 4-2 (AFRC, 1993) was used to calculate ME. Nutritional analyses were conducted at Lincoln University by the Animal and Food Sciences Group, using a Foss NIR Systems 5000 Rapid Content Analyser (Welltech Scientific Inc. 14600 Flint Lee Road, Unit-A, Chantilly, VA 20151 USA).

Equation 4-1 $\mathrm{CP} = \mathrm{N}\% * 6.25$

Where CP is crude protein.

Equation 4-2 $\mathrm{ME} = \mathrm{DOMD} * 0.16$

Where ME is metabolisable energy and DOMD is dry organic matter digestibility.

4.8.5 Nitrogen recovery of the pastures

To compare N recovery of ± N pastures, the amount of N (kg N ha^{-1}) removed in herbage at the end of each rotation was calculated using Equation 4-3. This was summed to estimate annual N removed by ±N pastures from N fertiliser and/or soil N. The difference in N recovered between + and −N pastures indicated the extent of deficiency in −N pastures. It also allowed the recovery of applied N fertiliser (kg N ha^{-1}) to be determined for the +N pastures.

Equation 4-3 $\text{Nitrogen recovery (kg N ha}^{-1}) = \mathrm{N}\% * \text{green material DM yield}$

4.9 Calculations

4.9.1 Thermal time accumulation

Mills *et al*. (2006), calculated thermal time accumulation of 'Wana' cocksfoot using a broken-stick threshold model (Jones and Kiniry, 1986) which employed hourly mean air temperature. For the growing period, base and optimum temperatures were derived by minimising the coefficient of variation. Different pasture grasses used in the current study might have different base and optimum temperature. However, to enable comparison of different grasses under the same conditions, and also to compare the results of the current study with the previous studies, a single base temperature of 3 °C and an optimum temperature of 23 °C were considered for all species. Thermal time was calculated from 30 days after sowing (on 16/11/2014, once seedlings number reached to a constant number) in the first year of study (2014/15). In the second year of study (2015/16), this started from 7/7/2015 at Ashley Dene and 10/6/2015 at Ladbrooks. Thermal time is accumulated on a daily basis from the mean hourly temperature using

Equation 2-2 (Section 2.2.1).

At Ladbrooks, a two stage broken stick models (Draper and Smith, 1998) were fitted to the relationship between accumulated DM production and Tt for each species/N treatment. Breaks indicate reduced growth due to the onset of moisture stress (Chapter 5). At Ashley Dene, this had five stages, due to more frequent periods of moisture stress. Wherever possible, a single regression line was fitted to mean DM production of + and −N for brome, perennial ryegrass and tall fescue. The linear regression lines fitted to cocksfoot were separated for + and −N treatments.

4.9.2 Mean daily growth rate

At the end of each rotation, daily growth rates (kg DM ha^{-1} d^{-1}) were estimated by dividing total dry matter production (kg DM ha^{-1}) by regrowth cycle period (d).

4.9.3 Potential Evapotranspiration

Potential evapotranspiration was calculated daily from hourly weather data from Broadfields meteorological station using Penman evapotranspiration potential (EP) as described by French and Legg (1979).

4.9.4 Potential soil moisture deficit (PSMD)

Equation 4-4) was used to calculate potential soil moisture deficit during a certain period of time (French and Leggs, 1979).

Equation 4-4 $\mathrm{PSMD}_i = \mathrm{PDSM}_{i-1} - EP_i + rainfall_i$

Where EP is Penman evapotranspiration potential and $PSMD_{i-1}$ is the PSMD on the earlier day (mm).

4.10 Statistical analysis

To allow the plants to utilize any background N at Ladbrooks (Table 4-3), the first N fertiliser was applied on 18/9/2015 at this site. To be able to compare the results from Experiment 1 with Experiment 2, the first N at Ashley Dene was applied on 21/9/2015 (Appendix F).

Before applying N, in the establishment year (2014/15), the number of seedlings and their root and shoot DM and also annual DM production at each site were analysed using the Latin square design. Total DM production in the subsequent year (2015/16), after N application, was analysed using a strip-plot design (for ±N treatments) at each site. DM yield and daily growth rates were also analysed for each destructive harvest using a Latin square design, before applying N fertiliser. In the second year (after N application), this was analysed as a strip-plot design with ±N as the rows and pasture species as the columns. Significant means were separated by Fishers protected least significant difference (LSD) at the 0.05 level. When interactions occurred, means were separated using the most conservative interaction LSD. In many cases, P values were slightly higher than 0.05 (for example P=0.052). In these cases, a trend was reported where the results were biologically meaningful and consistent with other results. Repeated measures analysis was used in Genstat 16.1 to investigate the effect of time on DM yield of each species in each destructive harvest.

A single base temperature of 3 °C, and an optimum temperature of 23 °C were allocated to all species (Mills, 2007). Temperature adjusted growth rates (kg DM $°Cd^{-1}$ ha^{-1}) were derived by regression of accumulated DM versus accumulated thermal time (Tt) for all ±N species treatments at the both sites using air temperature (Section 4.9.1).

In this chapter the agronomic results for DM production from a total of 14 regrowth periods for Experiment 1 at Ladbrooks (between 16/10/2014 and 10/07/2016) and eight regrowth periods for Experiment 2 at Ashley Dene (between 15/10/2014 and 7/07/2016) are reported. The first year results (2014/15) for each site, compared grass DM production and physiological responses to the different soil moisture status only. In the second year (2015/16), the combined effects of moisture and N are investigated.

4.11 Results

4.11.1 Seedling number

Experiment 1 at Ladbrooks

Figure 4-4 A shows the mean seedling number in 1 m of drill rows, 61 days after sowing (DAS) on 16/12/2014 (Section 4.8.1) at Ladbrooks. The mean number of perennial ryegrass seedlings (78.0 ± 6.88) was higher (P≤0.01) than brome and cocksfoot (31.5 and 37.5) and tall fescue was intermediate (57.8) (Figure 4-4 A). These represented 20 to 30% of the seed sown for brome, cocksfoot and tall fescue and 51.2% for perennial ryegrass.

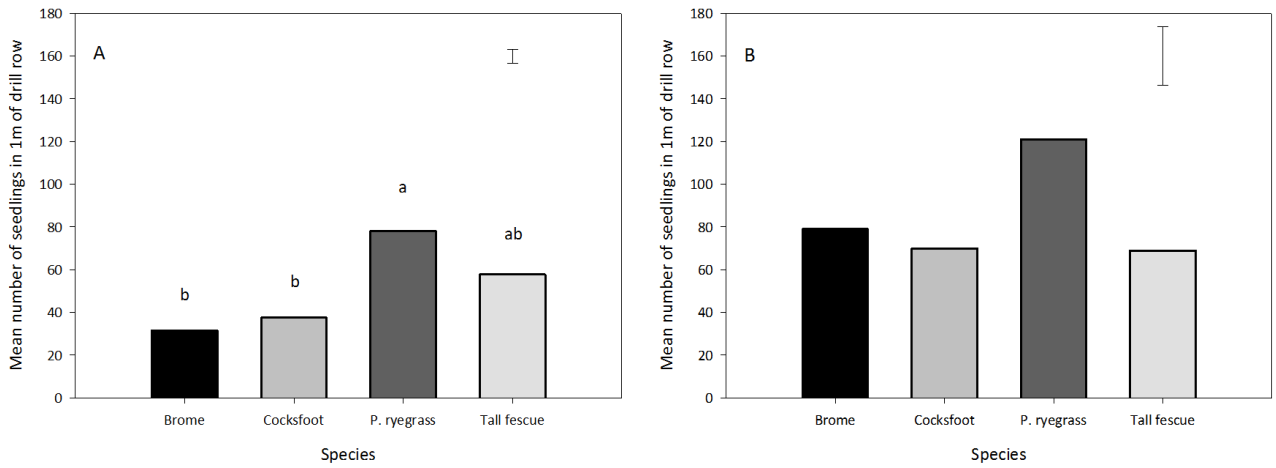


Figure 4-4 Mean number of seedlings for brome, cocksfoot, perennial ryegrass and tall fescue in 1 m of drill row at Ladbrooks (A) and Ashley Dene (B), Canterbury, New Zealand on 16/12/2014. The error bars show SEMs for species. Columns under same letters are not significantly different at P≤0.05. At Ashley Dene (B) there was no significant difference.

Experiment 2 at Ashley Dene

On 16/12/2014 (62 DAS), there was no difference (P=0.53) for the mean number of seedlings for all species at Ashley Dene. This ranged from 69.0 ± 27.5 for tall fescue to 120 for perennial ryegrass (Figure 4-4 B). Within the replicates, the mean number of seedlings in 1 m of drill row at Ashley Dene was more variable (CV%=34.2) than Ladbrooks (CV%=14.9). Field observations, suggest this resulted from variability in the stone content in the top soil layer of some plots at Ashley Dene with a Lismore stony soil (Section 4.5.2).

By 62 DAS at Ashley Dene, the number of seedlings/m represented 43% to 51% of the sown seeds for brome, tall fescue and cocksfoot and 80% for perennial ryegrass.

4.11.2 Seedling root and shoot dry weight

Experiment 1 at Ladbrooks

At 61 DAS (on 16/12/2014) at Ladbrooks, mean seedling root dry weight per plant for brome (0.0538 ± 0.00794 g) was higher (P≤0.01) than other species (Figure 4-5 A). The mean root dry weight of cocksfoot per plant (0.0150 g) was the lowest, but not different from perennial ryegrass (0.0328 g) or tall fescue (0.0271 g).

The average shoot dry weight per plant (Figure 4-5 A) for brome (0.211 ± 0.0323 g) was higher (P≤0.05) than cocksfoot (0.0521 g) and tall fescue (0.101 g) but not different from perennial ryegrass (0.127 g).

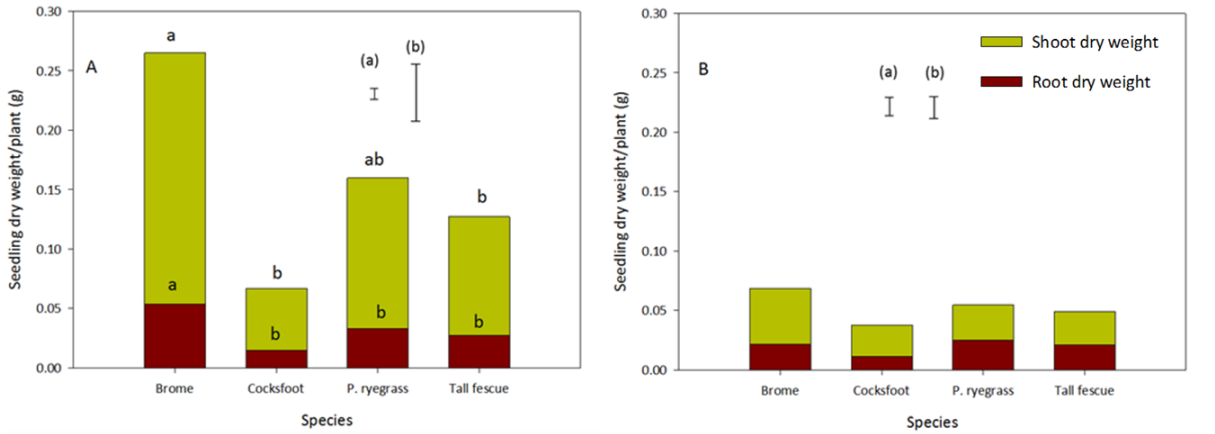


Figure 4-5 Mean seedling root and shoot dry weight per plant (g) for brome, cocksfoot, perennial ryegrass and tall fescue in 1 m of drill row on 16/12/2014 at Ladbrooks (A) and Ashley Dene (B), Canterbury, New Zealand. The error bars show SEMs for root (a) and shoot (b) dry weight at each site. At Ladbrooks (A), shoot and root dry weight columns under same letters are not significantly different at P≤0.05. At Ashley Dene (B) there was no significant difference.

Experiment 2 at Ashley Dene

On 16/12/2014 (62 DAS), at Ashley Dene, mean seedling root dry weight per plant was not different (P=0.542) and ranged from 0.0116 ± 0.0112 g for cocksfoot to 0.0342 g for brome (Figure 4-5 B). There was an indication (P=0.072) among species that the mean seedling shoot dry weight per plant differed. Mean seedling shoot dry weight at Ashley Dene ranged from 0.0269 ± 0.0134 g for cocksfoot to 0.0784 g for brome (Figure 4-5 B).

There was also an indication (P=0.067) of higher mean seedling root and shoot dry weight at Ladbrooks compared with Ashley Dene (Figure 4-5 B).

4.11.3 Seedling root/shoot ratio

Experiment 1 at Ladbrooks

By 61 DAS, mean root/shoot ratio at Ladbrooks was 0.288 ± 0.010 g and was not different (P=0.884) among species (Table 4-4).

Experiment 2 at Ashley Dene

By 62 DAS at Ashley Dene the mean root/shoot ratio was 0.487 ± 0.032 g and was not different (P=0.170) among species (Table 4-4).

Table 4-4 Mean root/shoot ratio of brome, cocksfoot, perennial ryegrass and tall fescue seedling grown at Ladbrooks and Ashley Dene, Canterbury, New Zealand on 16/12/2014 (61 and 62 DAS).

Species (S)	Ladbrooks	Ashley Dene
Brome	0.263	0.452
Cocksfoot	0.311	0.449
P. ryegrass	0.294	0.583
Tall fescue	0.283	0.464
Mean	0.288	0.487
P value	0.884	0.170

Table 4-4 shows, seedling root/shoot ratio was not different among species at each site.

4.11.4 Dry matter production

Experiment 1 at Ladbrooks

First year (2014/15)

In the establishment year (from 17/10/2014 to 9/6/2015 before the application of N fertiliser), total DM yield was not different (P=0.186) among the species at 4.34 ± 0.330 t ha^{-1}. Repeated measures analysis showed that time affected (P≤0.01) DM production of all species. On 7/1/2015 and 24/4/2015 DM production of all species was lower than the rest of the year (Figure 4-6 A and C). Dry matter production of all treatments in each destructive harvest is shown in Appendix G.

Second year (2015/16)

Annual DM of each species was not different (P=0.665) among species within +N and −N treatments in the second year of the study (from 10/6/2015 to 10/6/2016). However, total annual DM of all +N species was higher (P≤0.01) than −N treatment (Figure 4-6 B and D). Mean annual DM production of +N grasses was 19.8 ± 0.523 t ha^{-1}.

The first N fertiliser was applied in the second year on 18/9/2015 (Appendix F). However, DM yield of +N and −N treatments were similar until December 2015 (Figure 4-6 B and D). From 8/12/2015 to 10/6/2016, there was an increase (P≤0.01) in DM of all species in +N compared with −N treatments.

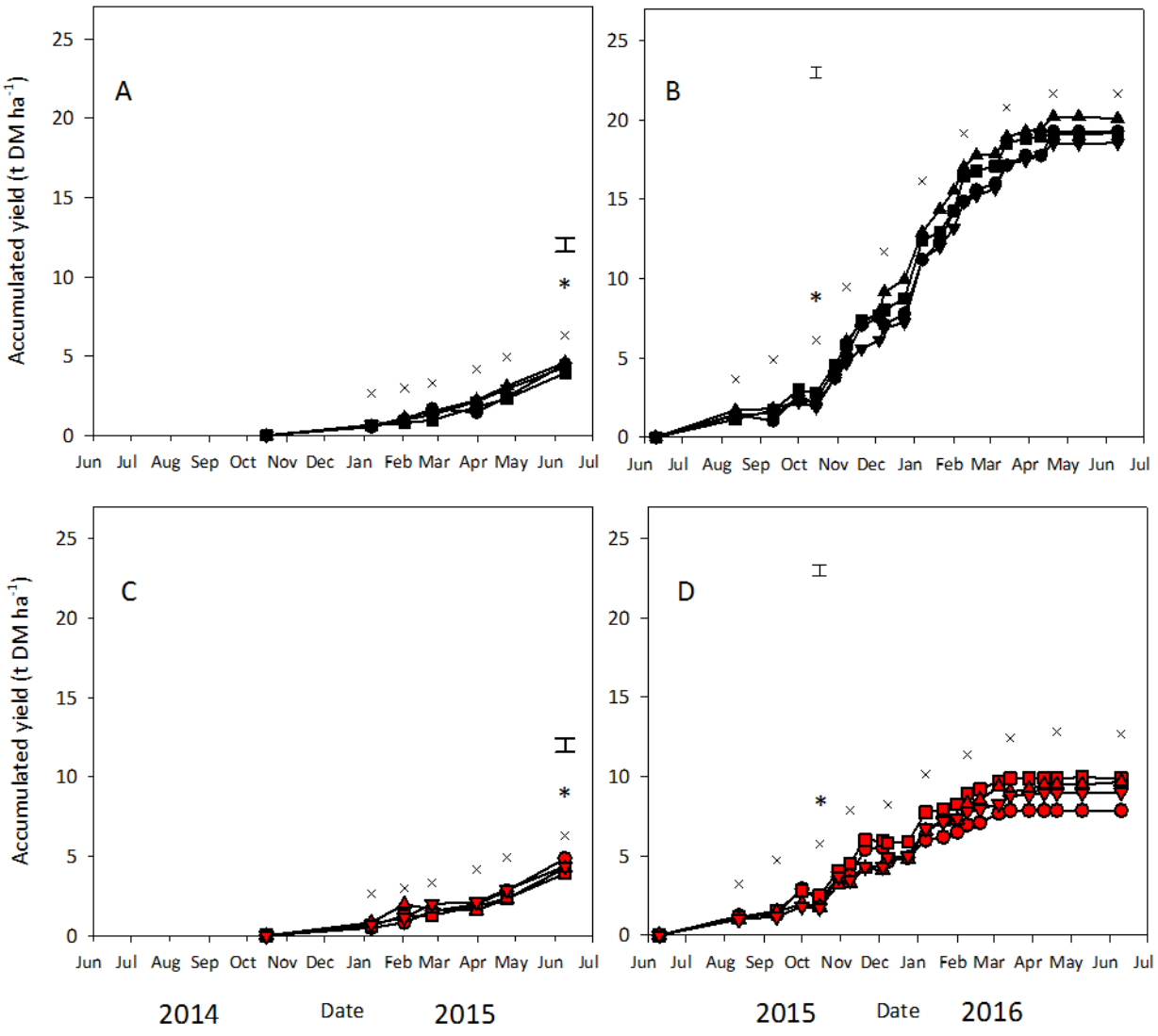


Figure 4-6 Accumulated dry matter (DM) by +N (black symbols) and −N (red symbols) monocultures of brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) over time, in 2014/15 (A and C) and 2015/16 (B and D) at Ladbrooks, Canterbury, New Zealand. The error bars are the highest LSDs when species treatments were different (P≤0.05) for DM production. The crosses (×) indicate the destructive harvest dates. Asterisks show where significant differences occurred (α≥0.05).

Experiment 2 at Ashley Dene

First year (2014/15)

In 2014/15 (16/10/2014-7/7/2015), before the application of N fertiliser, there was no difference (P=0.052) in total accumulated DM among the species (Figure 4-7 A and C). The average accumulated DM was 1.93 ± 0.116 t ha^{-1} (Figure 4-7 A and C).

On 7/7/2015, DM of brome and perennial ryegrass was 1.77 and 1.60 ± 0.121 t ha^{-1} respectively and higher (P≤0.05) than tall fescue (1.21 t ha^{-1}) with cocksfoot being intermediate (Figure 4-7 A and C).

Repeated measures analysis for the establishment year showed no interaction (P=0.068) between time and species treatments. There was an increase (P≤0.01) in DM of all species for 7/7/2015. This shows that, under the same conditions in the first year (2014/15), all species had a similar response to the environmental variables and accumulated the same amount of DM over this period of time.

Second year (2015/16)

Repeated measures analysis in the second year (from 8/7/2015 to 7/7/2016) showed that, on only one occasion there was an interaction (P≤0.001) between species and N in total DM. This occurred in the middle of January, once 85 mm of the summer rain increased DM production of +N cocksfoot, being 70% more than the other +N grass species.

In 2015/16, total annual DM production of cocksfoot in +N treatments was higher (P≤0.01) than all other species (Figure 4-7 B and D). Total DM of tall fescue, brome and perennial ryegrass was similar in ±N treatments. However, N application caused an increase (P≤0.01) in the annual DM yield of cocksfoot compared with −N treatments. Annual DM of +N pastures was 6.07 t ha^{-1} for cocksfoot compared with 3.31, 3.64 and 2.82 t ha^{-1} for brome, perennial ryegrass and tall fescue respectively (Figure 4-7 B and D).

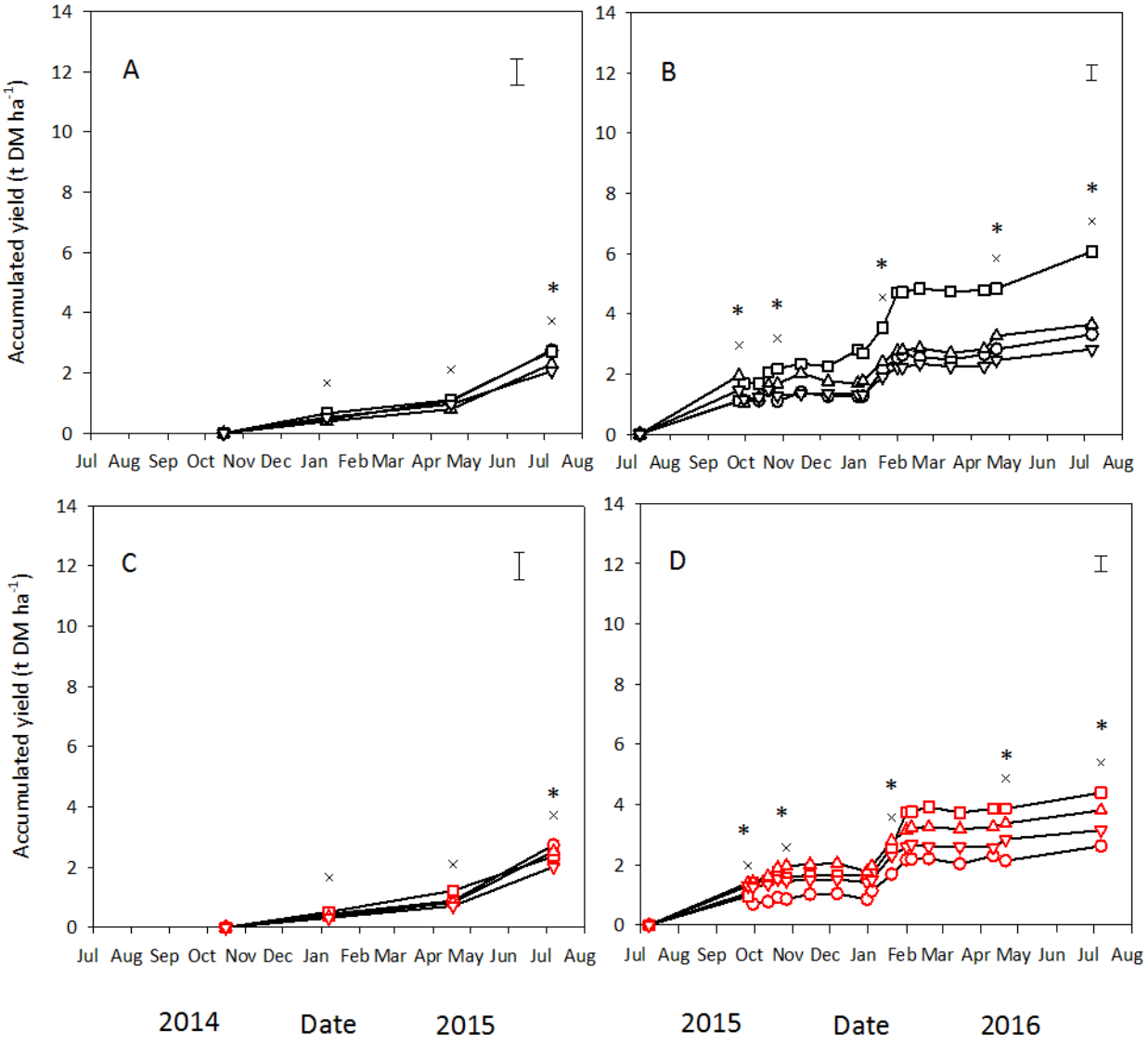


Figure 4-7 Accumulated dry matter (DM) by +N (black symbols) and −N (red symbols) monocultures of brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) over time, in 2014/15 (A and C), and 2015/16 (B and D) at Ashley Dene, Canterbury, New Zealand. The error bars are the highest LSDs when species treatments were different (P≤0.05) for DM production. The crosses (×) indicate the destructive harvest dates. Asterisks show where significant differences occurred (α≥0.05).

4.11.5 Mean daily growth rates

Experiment 1 at Ladbrooks

From 7/1/2015 to 9/6/2015 before the application of N fertiliser, mean daily growth rates of all species ranged from 5.87-31.50 kg DM ha^{-1} d^{-1}. Repeated measures analysis in the first year (2014/15) showed that, on 24/4/2015 mean daily growth rate of cocksfoot was lower (P≤0.01) than the other species. In June 2015 mean daily growth rate of brome was higher (P≤0.05) than other species (Figure 4-8 A and C).

The first application of N was on 18/9/2015 at Ladbrooks. From 8/12/2015 to 10/6/2016, there was no interaction (P=0.09) between species and N. Mean daily growth rates of all species were between 4.57-55.7 kg DM ha^{-1} d^{-1} for −N treatments, and 17.4-143.5 kg DM ha^{-1} d^{-1} for +N treatments (Figure 4-8 B and D). From 8/12/2015 to 10/6/2016, there was always an increase (P≤0.01) in mean daily growth rate of all species in +N compared with −N treatments. Thus, N application produced a similar response in each species at Ladbrooks.

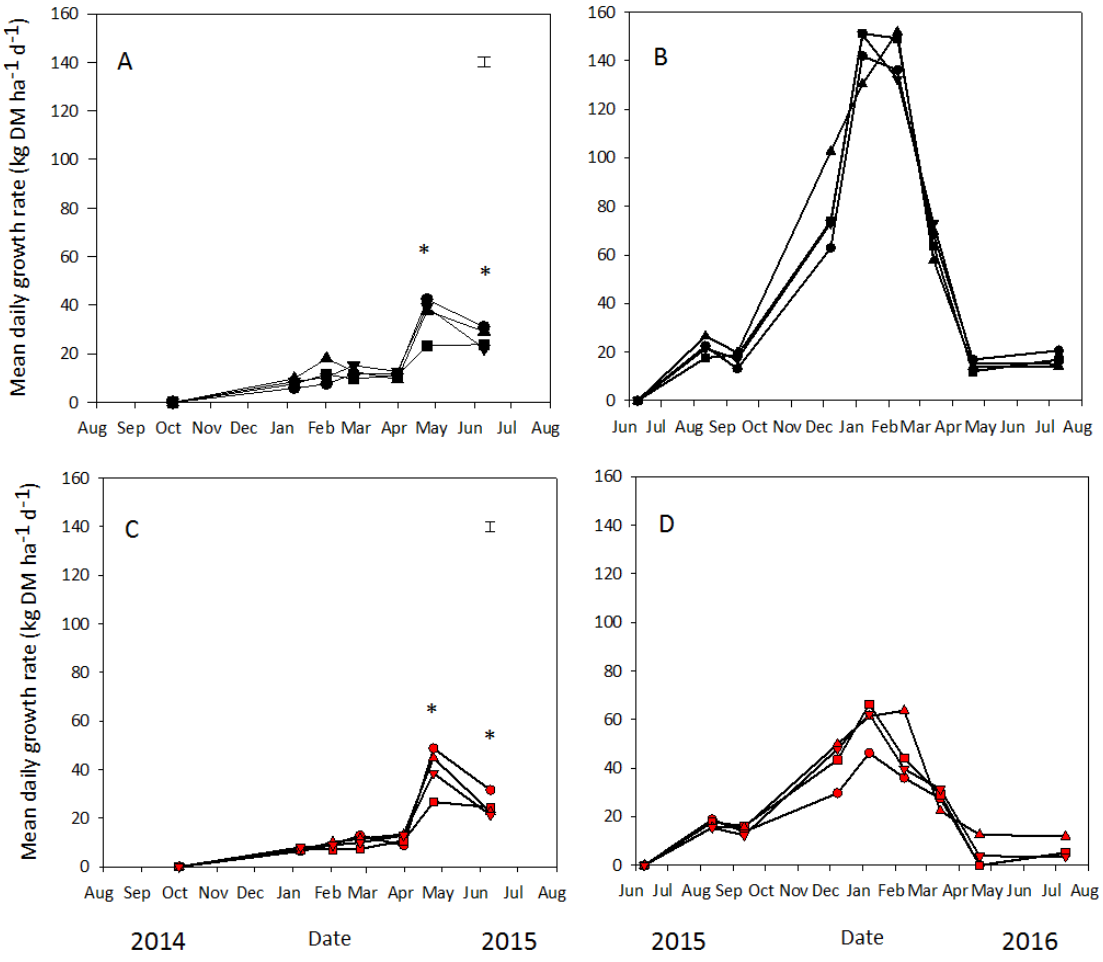


Figure 4-8 Mean daily growth rates (kg DM ha^{-1} d^{-1}) by +N (black symbols) and −N (red symbols) monocultures of brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) over time, in 2014/15 (A and C), and 2015/16 (B and D) at Ladbrooks, Canterbury, New Zealand. The error bars show SEMs when there was a significant (P≤0.05) difference among species. Asterisks show where significant differences occurred (α≥0.05).

Experiment 2 at Ashley Dene

Mean daily growth rates of all species ranged from 4.43-10.9 kg DM ha^{-1} d^{-1} in the establishment year (15/10/2014-7/7/2015). In the second year (26/9/2015-7/7/2016), mean daily growth rates of all species was between 5.13 to 28.9 kg DM ha^{-1} d^{-1} in ±N treatments (Figure 4-9 A and C).

In the second year (26/9/2015-7/7//2016), mean daily growth rates of all species were not different at the harvests on 26/9/2015 (15.2 ± 2.69 kg DM ha^{-1} d^{-1}) and 7/7/2016 (8.25 ± 0.142 kg DM ha^{-1} d^{-1}) in both ±N treatments (Figure 4-9 B and D). In +N treatments, in the second year, mean daily growth rate of perennial ryegrass and cocksfoot was higher (P≤0.01) than brome and tall fescue on 27/10/2015. In the summer time, from 20/1/2016 to 21/4/2016, mean daily growth rate of +N cocksfoot was higher (P≤0.01) than the other species (Figure 4-9 B).

From 26/9/2015-7/7/2016, in the −N treatment, mean daily growth rate ranged from the minimum of 5.13 kg DM ha^{-1} d^{-1} for brome (on 21/4/2016) to a maximum of 29.5 kg DM ha^{-1} d^{-1} for perennial ryegrass (on 27/10/2015). The maximum mean daily growth rate for −N tall fescue, cocksfoot and brome was 17.9 ± 1.53 kg DM ha^{-1} d^{-1} on 7/7/2015 (Figure 4-9 D).

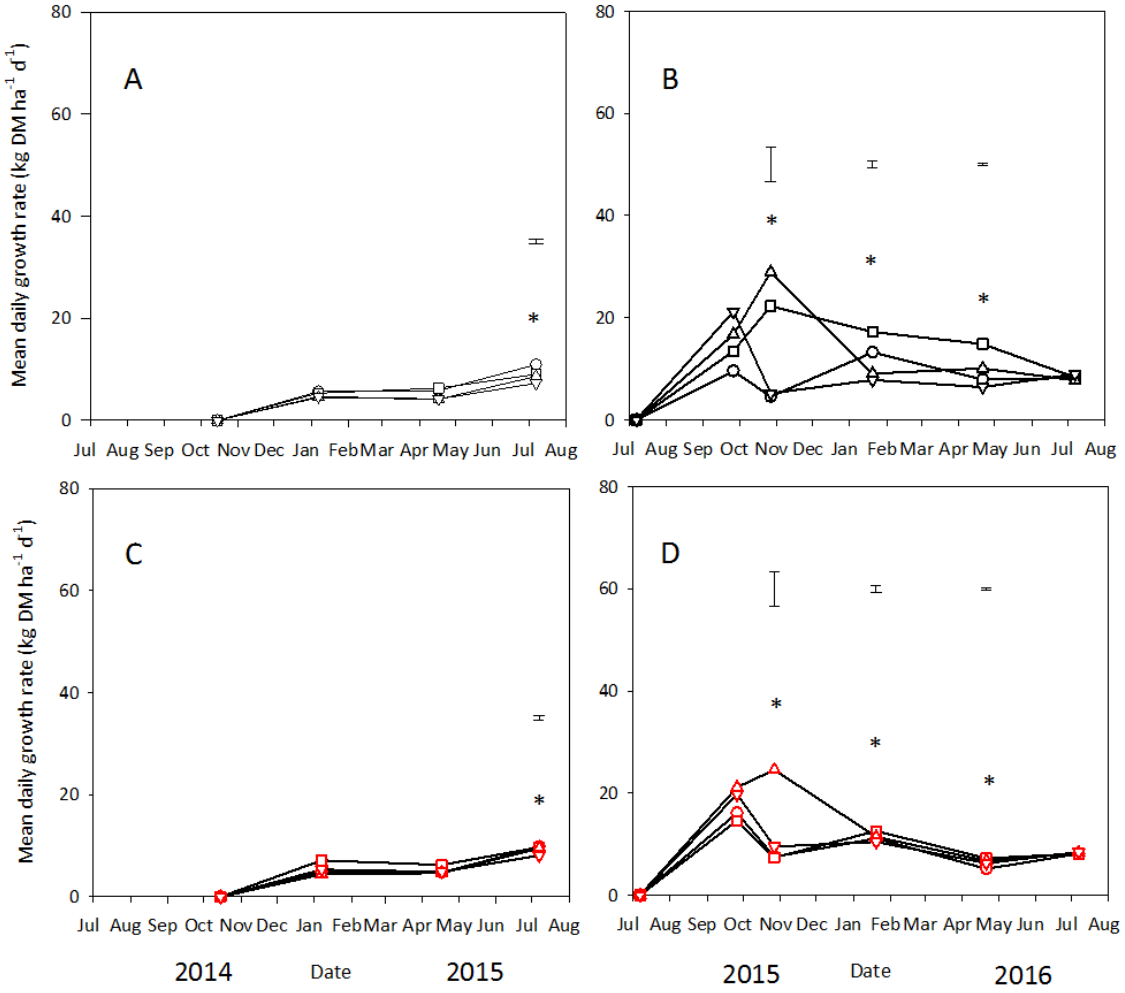


Figure 4-9 Mean daily growth rates (kg DM ha^{-1} d^{-1}) by +N (black) and −N (red) monocultures of brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) against time, in 2014/15 (A and C), and 2015/16 (B and D) at Ashley Dene, Canterbury, New Zealand. The error bars show SEM when there was a significant difference (P≤0.05) among species. Asterisks show where significant differences occurred (α≥0.05).

4.11.6 Botanical composition

Experiment 1 at Ladbrooks

Green grasses

From sowing until 7/7/2015, the total green grass fraction was not different (P=0.054) among species and ranged from 85.8% ± 2.82% for cocksfoot to 93.3% for brome (Figure 4-10 A and C). In the second year (8/7/2015-10/7/2016), the total green herbage fraction ranged from 97.2% ± 0.121% (for −N perennial ryegrass) to 99.5% for +N cocksfoot and was not different (P=0.056) among species and N treatments (Figure 4-10 B and D).

Over two years of study (from sowing to 10/7/2016), the total green herbage fraction was not different among N and species treatments. In the +N treatments, green herbages ranged from 93.7% for cocksfoot to 97.3% for tall fescue. In −N treatments, green grass material ranged from 93.3% for perennial ryegrass to 94.1% for cocksfoot.

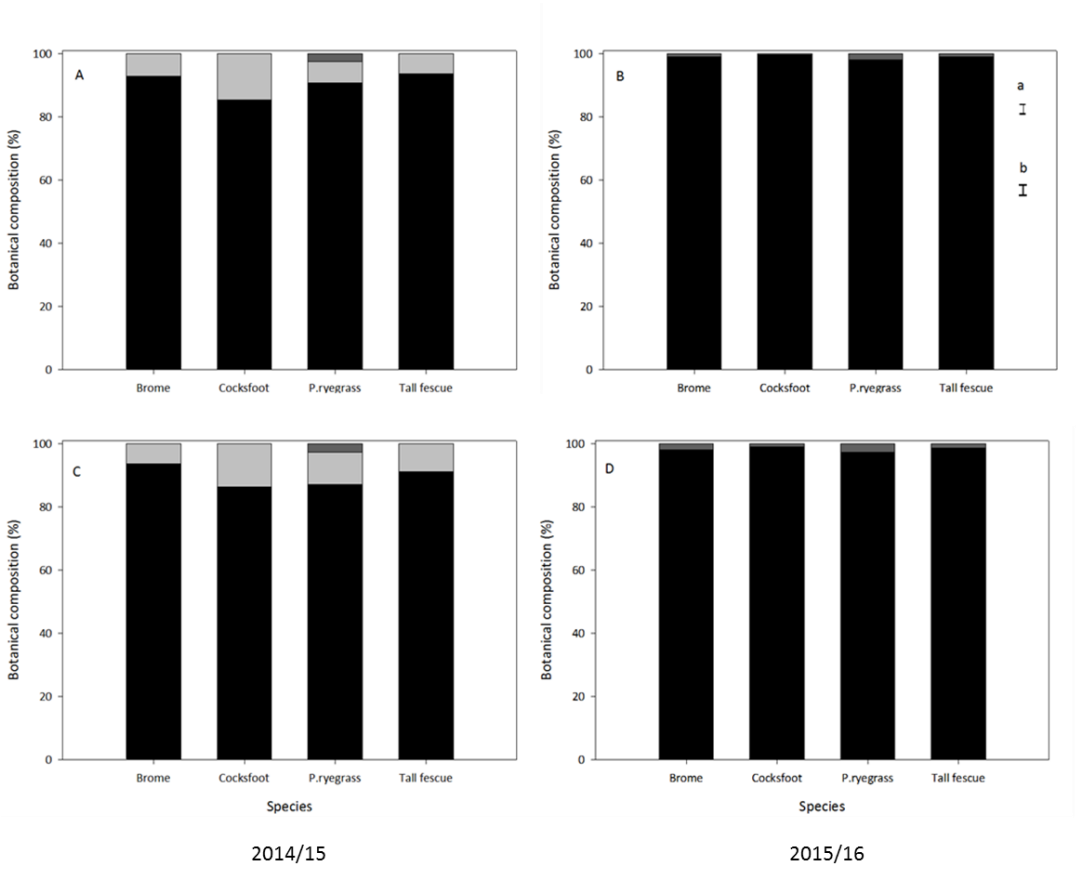


Figure 4-10 Botanical composition (grass (■), weeds (■) and dead material (■)) of brome, cocksfoot, perennial ryegrass (P. ryegrass) and tall fescue monocultures, in 2014/15 (A and C), and 2015/16 (B and D) at Ladbrooks, Canterbury, New Zealand. Treatments are +N (A and B) and −N (C and D). The error bars show SEMs for the grass fraction (a), weeds fraction (b) when there was a difference (P≤0.05) among species.

Weeds

Weeds were mostly present at the site (Plate 4-2) until 10/1/2015 which was 10 days after herbicide application at the site. The main weeds were fathen and wireweed. Weed species, contributed minimally to total annual DM yield throughout the experiment. From sowing until 9/6/2015, total DM of +N tall fescue, perennial ryegrass and brome contained 6.66-7.04% of weeds which was lower ($P<0.05$) than 14.6% for cocksfoot. At the same time, total DM contained 8.26% ± 3.76% of weeds in −N tall fescue, perennial ryegrass and brome and this was not different from −N cocksfoot (13.7%) (Figure 4-10 A and C).

Plate 4-2 Experiment site at Ladbrooks, Canterbury, New Zealand on 10/01/2015.

Dead material

From sowing until 7/7/2015, only perennial ryegrass contained 3% ± 0.316% of dead material (Figure 4-10 A and C) which was higher ($P<0.001$) than the other species (0%). In the second year (8/7/2015-10/7/2016), there was no difference ($P=0.057$) in total dead material among species and N treatments and this ranged from 0.47% ± 0.12% for +N cocksfoot to 2.78% for −N perennial ryegrass (Figure 4-10 B and D).

Experiment 2 at Ashley Dene

Green grasses

In the first year of the study (15/10/2014-7/7/2015) the green grass fraction of brome and perennial ryegrass comprised 92.9% ± 5.53% and 89.5% of the total yield which was higher ($P \leq 0.05$) than for tall fescue (77.2%). Cocksfoot green herbage was intermediate at 82.4% (Figure 4-11 A and C).

In the second year (8/7/2015 to 7/7/2016), the green material fraction was higher ($P < 0.001$) in +N compared with −N treatments. The green material proportion was not different ($P = 0.053$) among different species. In most cases, from 8/7/2015 to 7/7/2016, in both ±N treatments, brome contained the lowest and cocksfoot contained the highest proportion of green herbage. Total DM of +N brome contained 88.5% of green herbage compared with 77.6% for −N brome. In the second year, 95.7% of total DM of +N cocksfoot was green herbage which was more than the 93.3% for −N cocksfoot over the same period (Figure 4-11 B and D).

Over two years of the experiment (from sowing date to 7/7/2016), total accumulated green material was not different ($P = 0.22$) among all N and species treatments. Green grass fractions ranged from 78% for −N tall fescue to 91% for +N cocksfoot.

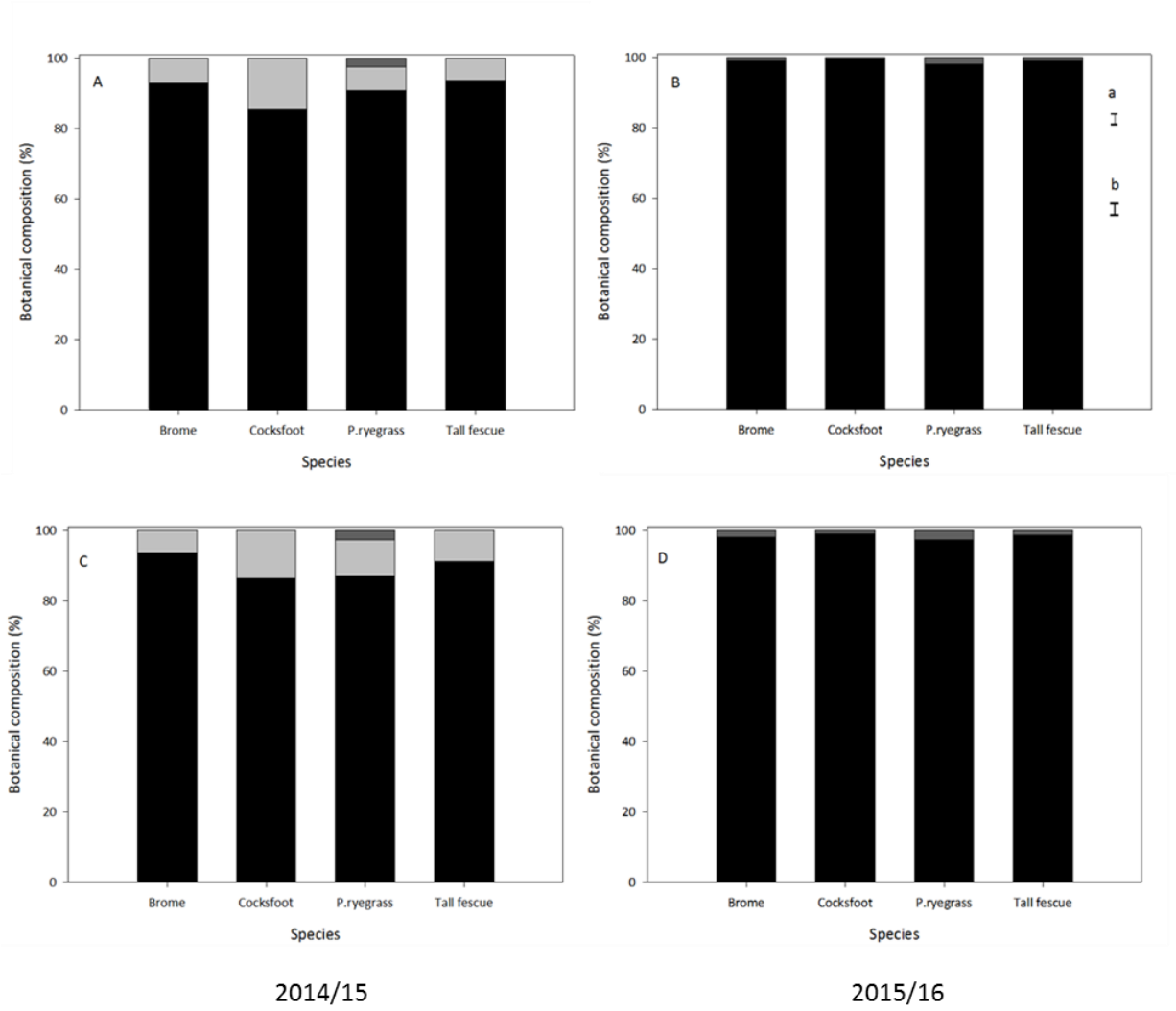


Figure 4-11 Botanical composition (grass (■), weeds (■) and dead material (■)) of brome, cocksfoot, perennial ryegrass and tall fescue monocultures, in 2014/15 (A and C), and 2015/16 (B and D) at Ashley Dene, Canterbury, New Zealand. Treatments are +N (A and B) and −N (C and D). The error bars show SEMs for the grass fraction (a) and weeds fraction (b) when there was a difference among species.

Weeds

Plate 4-3 shows the Ashley Dene site on 28/1/2015. In the first year of the study (7/1/2015-7/7/2015) total DM of tall fescue contained 22.8% ± 5.56% of weeds (mostly browntop and fathen) which was higher ($P<0.05$) than the 7.78% and 7.11% for perennial ryegrass and brome but was not different from cocksfoot (17.7%) (Figure 4-11 A and C).

Weeds were present at Ashley Dene until the second application of herbicide on 4/5/2015 (Section 4.7.5). In the second year (8/7/2015 to 7/7/2016), only 3.78% of total DM of +N tall fescue contained weeds (mostly browntop and wire weed) which was higher ($P<0.001$) than ±N brome, cocksfoot and perennial ryegrass plots (Figure 4-11 B and D).

Plate 4-3 Brome (A), cocksfoot (B), perennial ryegrass (C) and tall fescue (D), replicate three, at Ashley Dene, Canterbury, New Zealand on 28/1/2015. Weeds present at the site were mostly fathen and browntop.

Dead material

Total DM for cocksfoot, brome and tall fescue contained <1% ± 0.010% of dead material in the establishment year (7/1/2015-7/7/2015) which was lower (P<0.001) than the 2.70% for perennial ryegrass (Figure 4-11 A and C).

In the second year, from 8/7/2015 to 7/7/2016, dead material was higher (P<0.01) in −N treatments compared with +N treatments. In −N treatments, senesced material was 1.55 (for cocksfoot) to 2.22 times (for perennial ryegrass and tall fescue) more than the +N treatments (Figure 4-11 B and D). There was an indication (P=0.053) among species that the fraction of dead material differed. Overall, brome had the highest and cocksfoot had the lowest absolute amount of dead material in both ±N treatments. In +N treatments, this ranged from 4.33% ± 2.43% for cocksfoot to 11.5% for brome (Figure 4-11 B and D).

4.11.7 Nitrogen concentration (N%)

Experiment 1 at Ladbrooks

The annual measured N%, averaged over all harvest dates is represented in Table 4-5. From 16/10/2014 to 9/6/2015, before N application, there was an indication (P=0.088) among species that

mean annual N% differed. Mean annual N% was 2.61% for perennial ryegrass and was less than other species (Table 4-5).

In the second year (11/9/2015-10/7/2016), as expected, the mean annual N% was higher (P<0.001) in +N pastures. The mean annual N% for cocksfoot (2.76%), brome (2.72%) and tall fescue (2.62%) was higher (P<0.05) than perennial ryegrass pastures (2.50%).

Table 4-5 Mean annual N (%) of ±N green brome, cocksfoot, perennial ryegrass and tall fescue monocultures grown at Ladbrooks, Canterbury, New Zealand in 2014/15 and 2015/16.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	2.69	2.71	2.70	3.29	2.15	2.72 a
Cocksfoot	2.74	2.69	2.72	3.32	2.20	2.76 a
P. ryegrass	2.63	2.59	2.61	3.10	1.89	2.50 b
Tall fescue	2.72	2.60	2.66	3.28	1.96	2.62 a
Mean	2.70	2.65		3.25	2.05	
S	0.088			0.017		
N	0.420			<0.001		
S*N	-			0.484		
LSD (S)	-			0.159		
LSD (N)	-			0.110		
LSD (S*N)	-			-		

Note: S, N and S*N show the P values for species, nitrogen and species*nitrogen interactions. Means within a column with different letters are significantly different (LSD at the α≤0.05).

Before N application in the first year (from 16/10/2014 to 9/6/2015), there was only a difference among species in June 2015. Nitrogen% for cocksfoot was higher (P<0.05) than other species on 9/6/2015 (Figure 4-12 A).

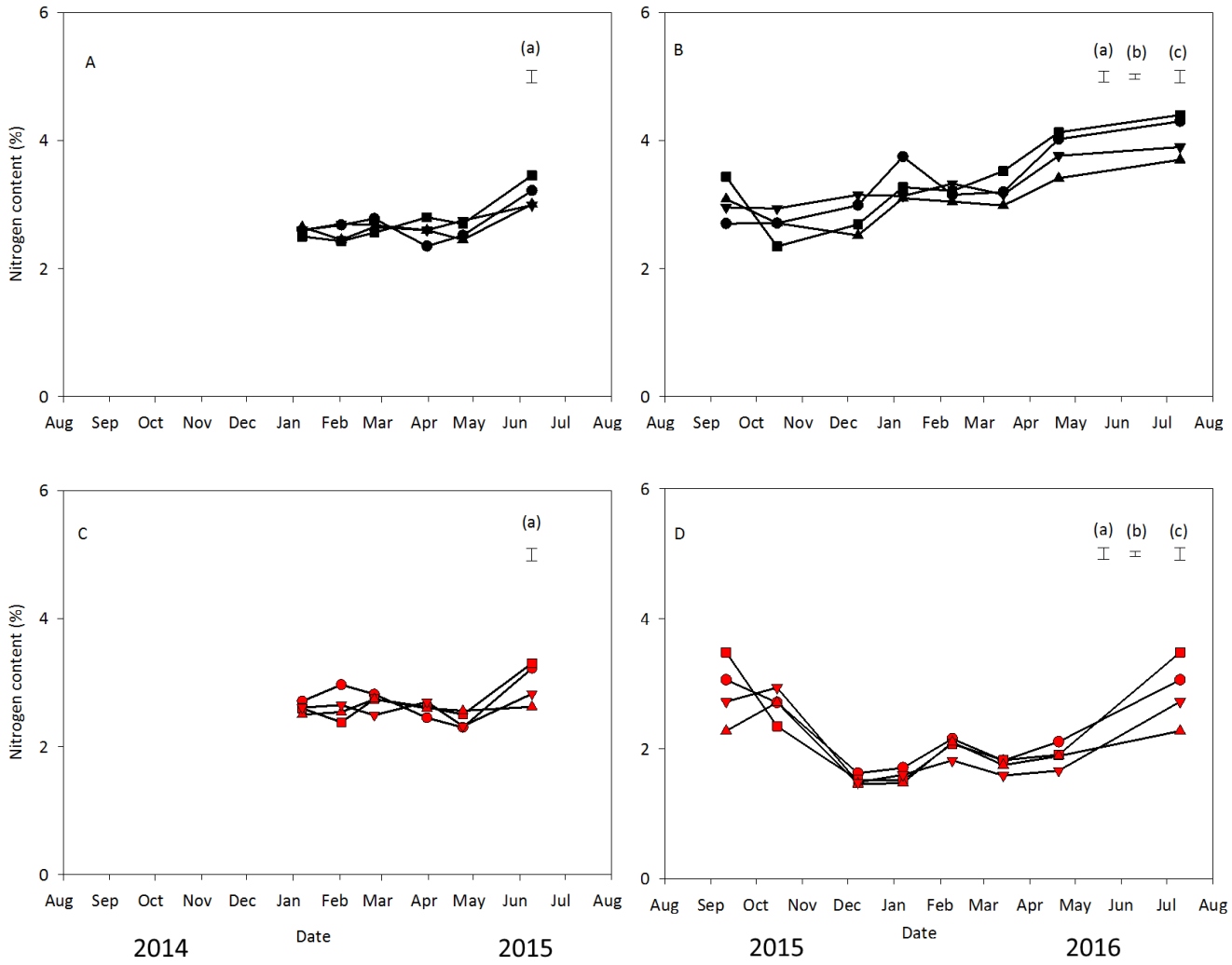


Figure 4-12 Measured N concentration (%) for green brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) herbage in +N (A and B) and −N (C and D) monocultures at Ladbrooks, Canterbury, New Zealand in 2014/15 (left) and 2015/16 (right). Error bars are maximum SEM for (a) species, (b) N and (c) species*N effects.

The mean N% was higher in the winter both in + and −N treatments than other seasons (Figure 4-12). The first N fertiliser was applied on 18/9/2015 at the site. However, N% was not different in + and −N treatments until 8/12/2015. From 8/12/2015 to 10/7/2016, N% was higher ($P<0.001$) in +N pastures compared with −N pastures at the end of each rotation. N% was different among species on two occasions. On 20/4/2016 and 10/7/2016, the average N% for cocksfoot and brome was higher ($P<0.05$) than other species.

Experiment 2 at Ashley Dene

From 7/1/2015 to 7/7/2015, before N application, the annual measured N%, averaged over all harvest dates are shown in Table 4-6. Mean annual N% was higher ($P<0.01$) for brome and cocksfoot and was

not different (P=0.910) for ±N treatments (Table 4-6). Mean annual N% ranged from 3.01% for perennial ryegrass to 3.49% for cocksfoot.

In the second year, there was no interaction (P=0.116) between N and species. From 26/9/2015 to 7/7/2016, mean annual measured N% was higher (P<0.05) for cocksfoot pastures. Application of N increased (P<0.001) mean annual measured N% for all species (Table 4-6).

Table 4-6 Mean annual N (%) of ±N green brome, cocksfoot, perennial ryegrass and tall fescue monocultures grown at Ashley Dene, Canterbury, New Zealand in 2014/15 and 2015/16.

				2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	3.39	3.30	3.35 a	3.47	2.90	3.18 b
Cocksfoot	3.46	3.51	3.49 a	3.54	3.37	3.45 a
P. ryegrass	3.06	2.96	3.01 b	3.26	2.66	2.96 c
Tall fescue	3.09	3.26	3.07 b	3.26	3.07	3.17 b
Mean	3.25	3.26		3.38	3.00	
S	0.002			0.016		
N	0.910			<.001		
S*N	-			0.116		
LSD (S)	0.168			0.267		
LSD (N)	-			0.164		
LSD (S*N)	-			-		

Note: S, N and S*N show the P values for species, nitrogen and species*nitrogen interactions. Means within a column with different letters are significantly different (LSD at the α≤0.05 level).

Repeated measures analysis showed that from 7/1/2015 to 7/7/2015 there was an interaction (P<0.01) between time and species. Mean N% was higher on 7/7/2015 than on other dates.

In the second year (2015/16), repeated measures analysis showed an interaction (P<0.01) between time and species. All species showed the lowest N% on 27/10/2015. N% for perennial ryegrass was the lowest on 7/7/2016.

On two occasions on 26/9/2015 and 7/7/2016, N application did not affect N%.

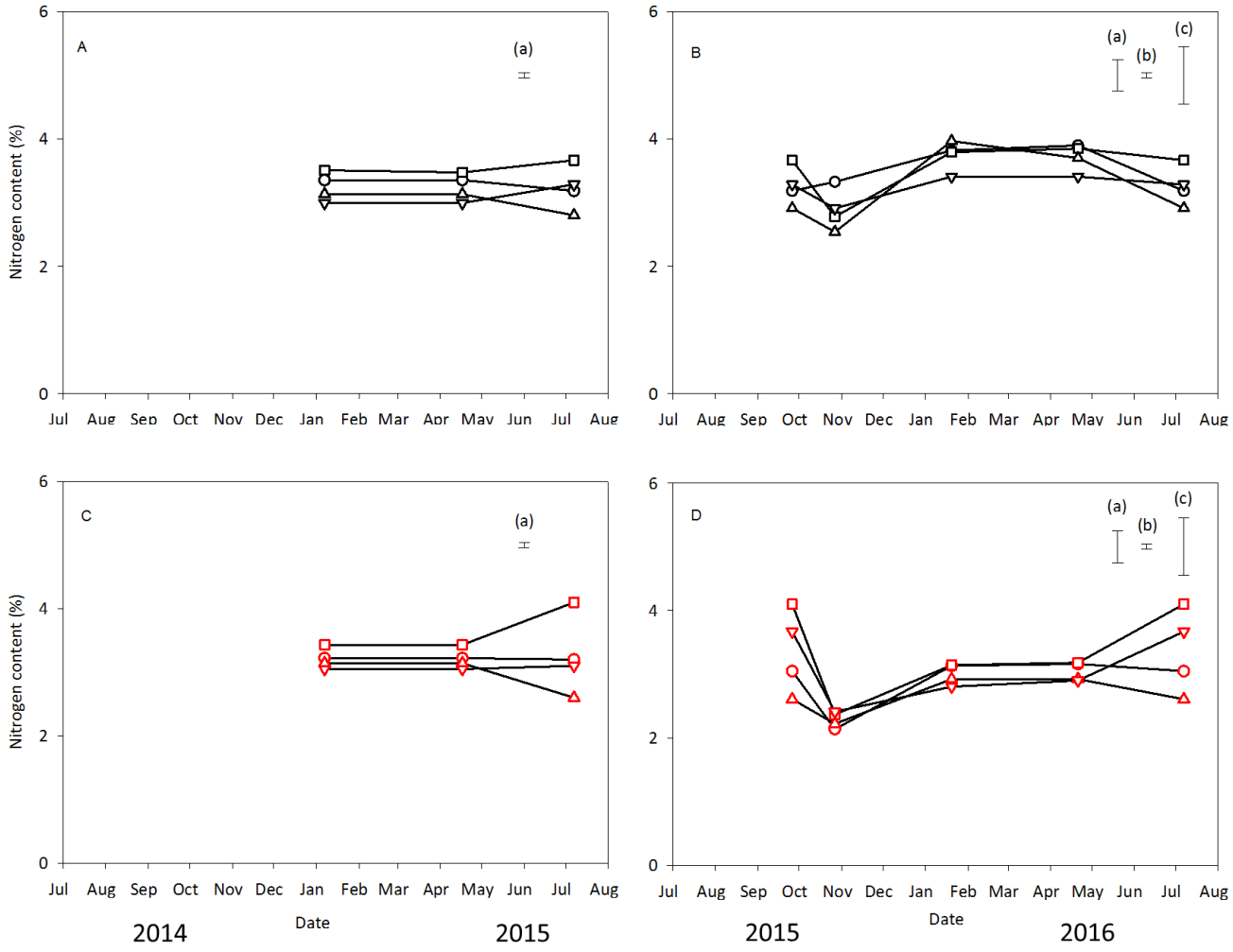


Figure 4-13 Measured N concentration (%) for green brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) in +N (A and B) and −N (C and D) monocultures at Ashley Dene, Canterbury, New Zealand in 2014/15 (left) and 2015/16 (right). Error bars are maximum SEM for (a) species, (b) N and (c) species*N effects.

4.11.8 Total nitrogen recovery by the pastures

Experiment 1 at Ladbrooks

Total N recovery by the pastures was calculated by the method described at Section 4.8.5. In the first year of study (2014/15), before application of N, total N recovery at Ladbrooks was 121 ± 5.48 kg ha^{-1} and was not different (P=0.327) among species (Table 4-7).

In the second year (2015/16), as expected, application of 900 kg N ha^{-1} (in eight split applications from 18/9/2015 to 20/4/2016), caused an increase (P<0.001) in N recovery of the pastures but this was not different (P=0.942) among species treatments (Table 4-7).

In 2015/16, an additional 435 ± 17.17 kg N ha^{-1} was removed from green herbage by the +N pastures in excess of soil mineral N supply as quantified by the −N pastures. Thus, 48% of the 900 kg N ha^{-1} yr^{-1} applied was accounted for. The recovery values are only valid for green harvested herbage and does not account for N in residual biomass below 3.0 cm, roots, amount of N in other pasture components or remaining soil N reserves. In addition, it has been shown previously that DM responses may occur up to six months after N application (Peri *et al*., 2002).

Table 4-7 Mean annual N recovery of ±N green brome, cocksfoot, perennial ryegrass and tall fescue monocultures grown at Ladbrooks, Canterbury, New Zealand in 2014/15 and 2015/16.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	137	139	138	633	174	403
Cocksfoot	117	111	114	637	207	422
P. ryegrass	124	109	116	600	179	389
Tall fescue	119	114	117	601	170	386
Mean	124	118		618	183	
S	0.327			0.942		
N	0.56			<0.001		
S*N	-			-		
LSD (S)	-			-		
LSD (N)	-			30.9		
LSD (S*N)	-			-		

Note: S, N and S*N show the P values for species, nitrogen and species*nitrogen interactions. Means within a column with different letters are significantly different (LSD at the α≤0.05 level).

Experiment 2 at Ashley Dene

In the first year of the study (2014/15), before application of N, mean total N recovery was 76.1 ± 1.76 kg ha^{-1} for brome and cocksfoot which was higher (P<0.01) than 56.7 ± 2.9 kg ha^{-1} for perennial ryegrass and tall fescue at Ashley Dene (Table 4-8).

Application of 500 kg N ha^{-1} in the second year (2015/16), caused an interaction (P<0.01) between species and N in total N recovery. N application increased total N recovery by brome, cocksfoot and perennial ryegrass but did not affect total N recovery by tall fescue pastures.

In 2015/16, mean annual N recovery by ±N cocksfoot was higher (P<0.01) than other species (Table 4-8). An addition of 20 and 38 kg N ha^{-1} was removed from green herbage by the +N brome and perennial ryegrass in excess of soil mineral N supply as quantified by the −N pastures. These only account for 4% and 8% of the 500 kg N ha^{-1} applied. In comparison, N recovery by +N cocksfoot pasture was 73 kg N ha^{-1}, which accounts for almost 15% of additional N application.

Table 4-8 Mean annual N recovery of ±N green brome, cocksfoot, perennial ryegrass and tall fescue monocultures grown at Ashley Dene, Canterbury, New Zealand in 2014/15 and 2015/16.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	81.1	74.9	78 a	124	86.5	105 b
Cocksfoot	75.5	72.9	74 a	193	120	157 a
P. ryegrass	55.5	59.4	57 b	128	108	118 b
Tall fescue	49	62.7	56 b	107	119	113 b
Mean	65	67		138	109	
S	0.008			0.002		
N	0.668			<0.001		
S*N	0.629			0.002		
LSD (S)	13.07			21.8		
LSD (N)	-			12.6		
LSD (S*N)	-			26.5		

Note: S, N and S*N show the P values for species, nitrogen and species*nitrogen interactions. Means within a column with different letters are significantly different (LSD at the α≤0.05 level).

4.11.9 Total crude protein (CP) yield

Experiment 1 at Ladbrooks

Crude protein (CP) yield was determined from the green harvested grasses. In the first year of the study (16/10/2014-9/6/2015), there was no difference (P=0.098) among species in total CP. Mean annual CP ranged from 0.717 ± 0.083 t CP ha^{-1} yr^{-1} for perennial ryegrass to 0.950 t CP ha^{-1} yr^{-1} for brome (Table 4-9).

In the second year (11/9/2015-10/7/2016), there was no interaction (P=0.223) between N and species in total CP yield (Table 4-9). Nitrogen increased (P≤0.001) crude protein of all species. Mean annual CP yield of +N treatments was 3.42 ± 0.17 t CP ha^{-1} yr^{-1} which was three times higher (P<0.001) than −N treatments (1.23 ± 0.17 t CP ha^{-1} yr^{-1}).

Table 4-9 Annual crude protein (CP) yield (t CP ha^{-1} yr^{-1}) of ±N brome, cocksfoot, perennial ryegrass and tall fescue monocultures grown at Ladbrooks, Canterbury, New Zealand in 2014/15 and 2015/16.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	0.958	0.941	0.946	3.48	1.29	2.39
Cocksfoot	0.797	0.759	0.778	3.29	1.32	2.31
P. ryegrass	0.784	0.650	0.717	3.44	1.28	2.36
Tall fescue	0.871	0.739	0.805	3.45	1.03	2.24
Mean	0.853	0.772		3.42	1.23	
S	0.098			0.823		
N	-			<0.001		
S*N	-			-		
LSD (S)	-			-		
LSD (N)	-			0.231		
LSD (S*N)	-			-		

Note: S and N show the P values for species and nitrogen treatments.

Individual regrowth cycle CP yields (kg CP ha^{-1}) in 2014/15 and 2015/16 are shown in Figure 4-14. In the establishment year (7/1/2015-9/6/2015), pasture CP was higher in late March and June 2015. In this period, pastures CP ranged from 30.1 kg CP ha^{-1} (in 7/1/2015) to 430 kg CP ha^{-1} (in 9/6/2015) (Figure 4-14 A and C). Repeated measures analysis for each destructive harvest showed an interaction between time and species on two occasions. On 7/1/2016 CP for perennial ryegrass (79.8 ± 11.6 kg CP ha^{-1}) was higher ($P<0.001$) than other species. Also on 9/6/2015, CP for brome (431 ± 40.9 kg CP ha^{-1}) was higher ($P<0.01$) than other grasses which was mostly due to its higher production during the winter (Figure 4-14 A).

In the second year (2015/16), CP was higher in +N treatments compared with −N treatments (Figure 4-14 B and D). CP yields (kg CP ha^{-1}) of +N pastures ranged from 150 (on 15/10/2015) to 1050 (on 7/1/2016). The minimum CP was found in winter or when no N was applied. In −N pastures, CP fluctuated in different seasons. The CP yield of the −N pastures ranged from 63.3 kg CP ha^{-1} (on 10/7/2016) to 337± 20 kg CP ha^{-1} (on 11/9/2015) (Figure 4-14 D).

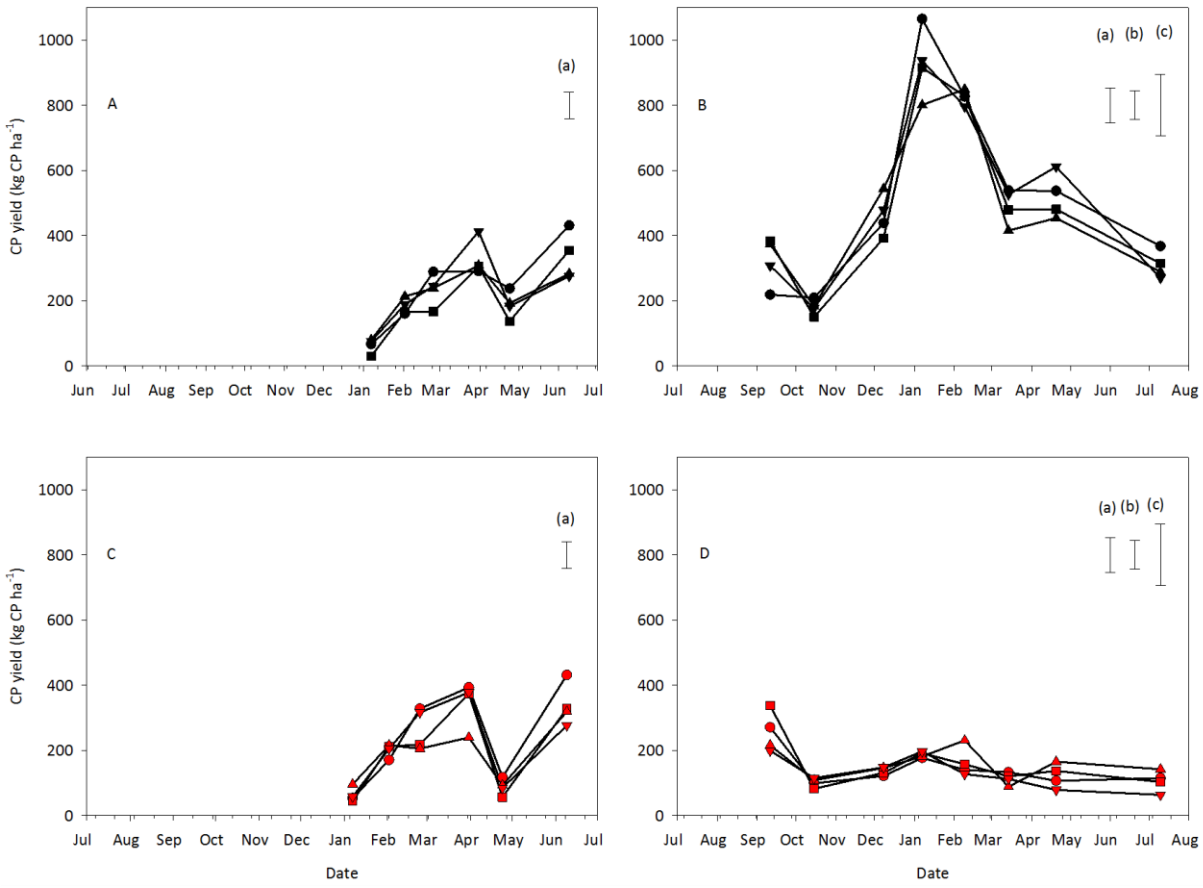


Figure 4-14 Crude protein (CP) yield (kg CP ha^{-1}) for monocultures of brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼), +N (A and B) and −N (C and D) in 2014/15 and 2015/16 grown at Ladbrooks, Canterbury, New Zealand. Error bars are maximum SEM for (a) species, (b) N and (c) species*N interactions in each year.

Experiment 2 at Ashley Dene

From 7/1/2015 to 7/7/2015, total CP yield was higher (P<0.01) for brome and cocksfoot. Total CP ranged from 0.335 t CP ha^{-1} for tall fescue to 0.470 t CP ha^{-1} for brome (Table 4-10).

In the second year (8/7/2015 to 7/7/2016), there was an interaction (P<0.05) between N and total CP yield of brome and cocksfoot. Total CP for cocksfoot was higher (P<0.01) than other species (Table 4-10). Except tall fescue, N increased (P<0.001) CP yield of other pastures (Table 4-10).

Table 4-10 Annual crude protein (CP) yield (t CP ha^{-1} yr^{-1}) of ±N brome, cocksfoot, perennial ryegrass and tall fescue monocultures grown at Ashley Dene, Canterbury, New Zealand in 2014/15 and 2015/16.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	0.490	0.450	0.470 a	0.76	0.52	0.639 b
Cocksfoot	0.458	0.451	0.454 a	1.20	0.75	0.975 a
P. ryegrass	0.342	0.361	0.351 b	0.79	0.66	0.723 a
Tall fescue	0.292	0.292	0.335 b	0.66	0.73	0.693 a
Mean	0.396	0.389		0.853	0.665	
S	0.008			0.002		
N	0.683			<0.001		
S*N	-			0.002		
LSD (S)	0.08			0.077		
LSD (N)	-			0.138		
LSD (S*N)	-			0.165		

Note: S and N show the P values for species and nitrogen. Means within a column with different letters are significantly different (LSD at the $\alpha \leq 0.05$ level).

Repeated measures analysis in the first year (7/1/2015 to 7/7/2015) shows that, only on 7/7/2015, CP for brome was higher ($P<0.01$) than other species (Figure 4-15).

In the second year, except for the end of October 2015 and early July 2016, +N cocksfoot always had higher CP compared with other grasses (Figure 4-15). CP for both +N (179 ± 12.1 kg CP ha^{-1}) and −N (35.9 ± 12.2 kg CP ha^{-1}) pastures was the lowest ($P<0.01$) on 27/10/2015. Among +N pastures, CP for tall fescue was lower ($P<0.01$) than other grasses during summer (Figure 4-15). In −N pastures, CP was highest on 26/9/2015 and lowest on 27/10/2015.

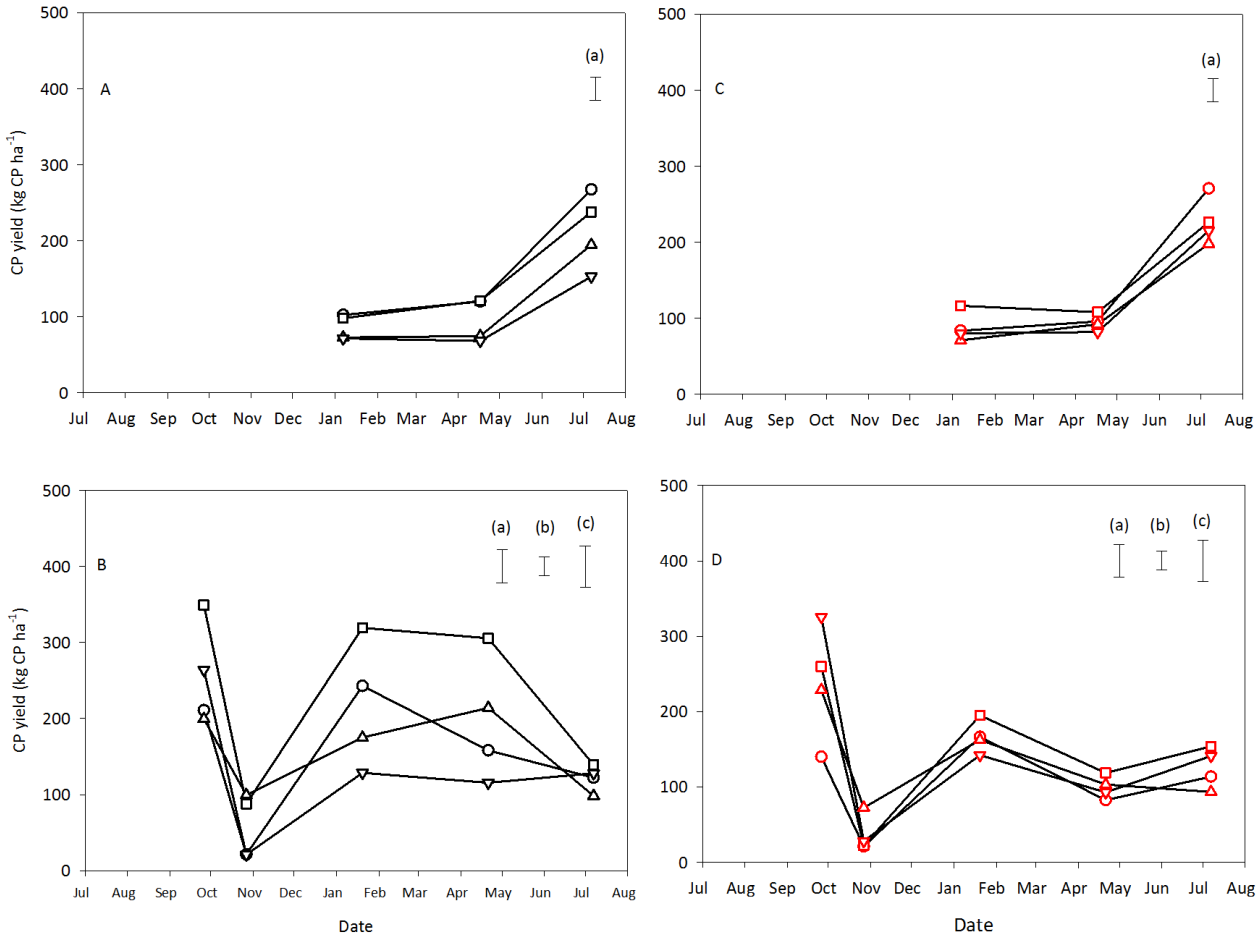


Figure 4-15 Crude protein (CP) yield (kg CP ha^{-1}) for monocultures of +N (A and B) and −N (C and D) brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) in 2014/15 and 2015/16 grown at Ashley Dene, Canterbury, New Zealand. Error bars are SEM for (a) species effects, (b) N effects and (c) species*N interactions of the total crude protein.

4.11.10 Metabolisable energy (ME) yield

Experiment 1 at Ladbrooks

From 7/1/2015 to 9/6/2015, annual ME for perennial ryegrass and tall fescue was higher ($P<0.05$) than cocksfoot with brome being an intermediate (Table 4-11).

In the second year, from 10/6/2015 to 10/7/2016, total annual ME of perennial ryegrass was higher ($P<0.01$) than other species (Table 4-11). Nitrogen application increased ($P<0.001$) the total ME for all species, but there was no interaction ($P=0.397$). Application of N caused a 100% (cocksfoot and perennial ryegrass) to 150% (tall fescue and brome) increase in ME predominantly due to the increase in yield for different species.

Table 4-11 Annual metabolisable energy (ME) yield (GJ ha^{-1} yr^{-1}) from ±N monoculture brome, cocksfoot, perennial ryegrass and tall fescue in 2014/15 and 2015/16 grown at Ladbrooks, Canterbury, New Zealand.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	79	70.2	74.6 bc	198	80.6	139 b
Cocksfoot	65.9	70.4	68.1 c	202	101	151 b
P. ryegrass	91.4	85	88.2 a	228	119	173 a
Tall fescue	82.6	81	81.8 ab	209	88.7	149 b
Mean	79.7	77		209	97.1	
S	0.016			0.002		
N	0.439			<.001		
S*N	-			0.397		
LSD (S)	11.4			13.3		
LSD (N)	-			9.25		
LSD (S*N)	-			-		

Note: S and N show the P values for species and nitrogen treatments. Means within a column with different letters are significantly different (LSD at the $\alpha \leq 0.05$ level).

Figure 4-16 shows metabolisable energy (ME) content (MJ/kg DM) of ±N brome, cocksfoot, perennial ryegrass and tall fescue in 2014/15 and 2015/16 at Ladbrooks. ME for brome was lower and for perennial ryegrass was higher than other species in both 2014/15 and 2015/16. In most cases, there were no differences in ME content of tall fescue and cocksfoot in the +N treatment. In −N treatment however, from September 2015 to March 2016, ME of tall fescue was lower than cocksfoot. In 2014/15, ME ranged from 10.6 to 11.7 MJ/kg DM during the year. In 2015/16, ME for all species was lower in the summer and higher in the spring and autumn (Figure 4-16).

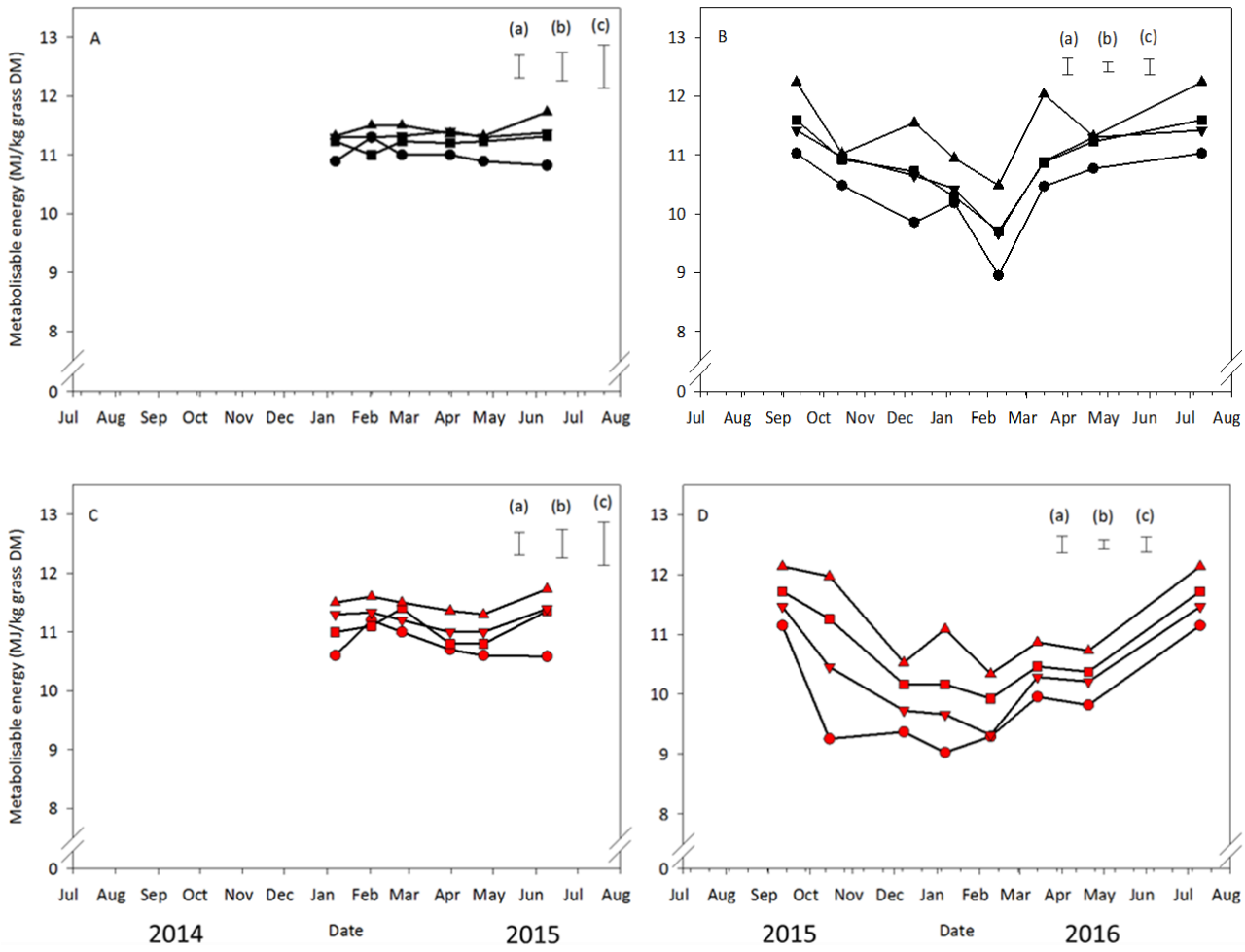


Figure 4-16 Metabolisable energy (ME) content (MJ/kg grass DM) of +N (A and B) and −N (C and D) brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) in 2014/15 and 2015/16 monocultures grown at Ladbrooks, Canterbury, New Zealand. Error bars are SEM for (a) species, (b) N and (c) species*N interactions of the total metabolisable energy.

In the second year, the maximum and minimum of ME were similar in both +N (8.95-12.2 MJ/kg DM) and −N (9.02-12.1 MJ/kg DM) treatments but at the minimum level for a longer period of time (October 2015-March 2016) in −N pastures. In most cases, ME of perennial ryegrass was higher and also fluctuated more than other species and ranged from 10.7 ± 0.231 MJ/kg DM in January and February to 12.1 ± 0.10 MJ/kg DM in March and July 2016 in +N treatment. In −N perennial ryegrass, the maximum ME was in the winter time, at 12 MJ/kg DM.

Over two years of study, in +N cocksfoot and tall fescue, ME ranged from a minimum of 9.92 MJ/kg DM in February 2016 to a maximum of 11.7 MJ/kg DM in winter 2015 and 2016. In −N cocksfoot and tall fescue, ME levelled off at the minimum level for a longer period of time (10.3 ± 0.160 for cocksfoot and 9.93 ± 0.311 MJ/kg DM for tall fescue) from October 2015 to April 2016.

Experiment 2 at Ashley Dene

In 2014/15 (from 7/1/2016 to 7/7/2016), mean total ME was 27.2 ± 8.62 GJ ha^{-1} yr^{-1} in 2014/15 and was not different (P=0.103) among species (Table 4-12).

In the second year (2015/ 16), there was an interaction (P≤0.001) between N and species in total ME. This shows that annual ME for brome and cocksfoot was affected by N application more than other species. Mean annual ME of perennial ryegrass and cocksfoot was higher (P≤0.001) than brome and tall fescue (Table 4-12).

Table 4-12 Annual metabolisable energy (ME) yield (GJ ha^{-1} yr^{-1}) for monocultures of ±N brome, cocksfoot, perennial ryegrass and tall fescue in 2014/15 and 2015/16 grown at Ashley Dene, Canterbury, New Zealand.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	31.4	28.9	30.2	38.5	31.1	34.8 b
Cocksfoot	27.6	28.8	28.2	60.1	40.0	50.1 a
P. ryegrass	27.0	28.9	28.0	45.6	47.5	46.5 a
Tall fescue	20.9	24.3	22.6	38.0	41.9	39.9 b
Mean	26.7	27.7		45.6	40.1	
S	0.103			<.001		
N	0.635			0.005		
S*N	-			<.001		
LSD (S)	-			5.70		
LSD (N)	-			3.49		
LSD (S*N)	-			7.08		

Note: S, N and S*N show the P values for species, N and species*N interactions. Means within a column with different letters are significantly different (LSD at the α≤0.05 level).

In both +and −N treatments, ME was maximum in the middle of Sep 2015 and decreased gradually to the minimum from Nov to Apr 2016 (Figure 4-17). On 20/1/2016 and 21/4/2016, ME for cocksfoot was higher (P<0.001) than other species. In winter (2710/2015), ME of brome was higher (P≤0.05) than other species.

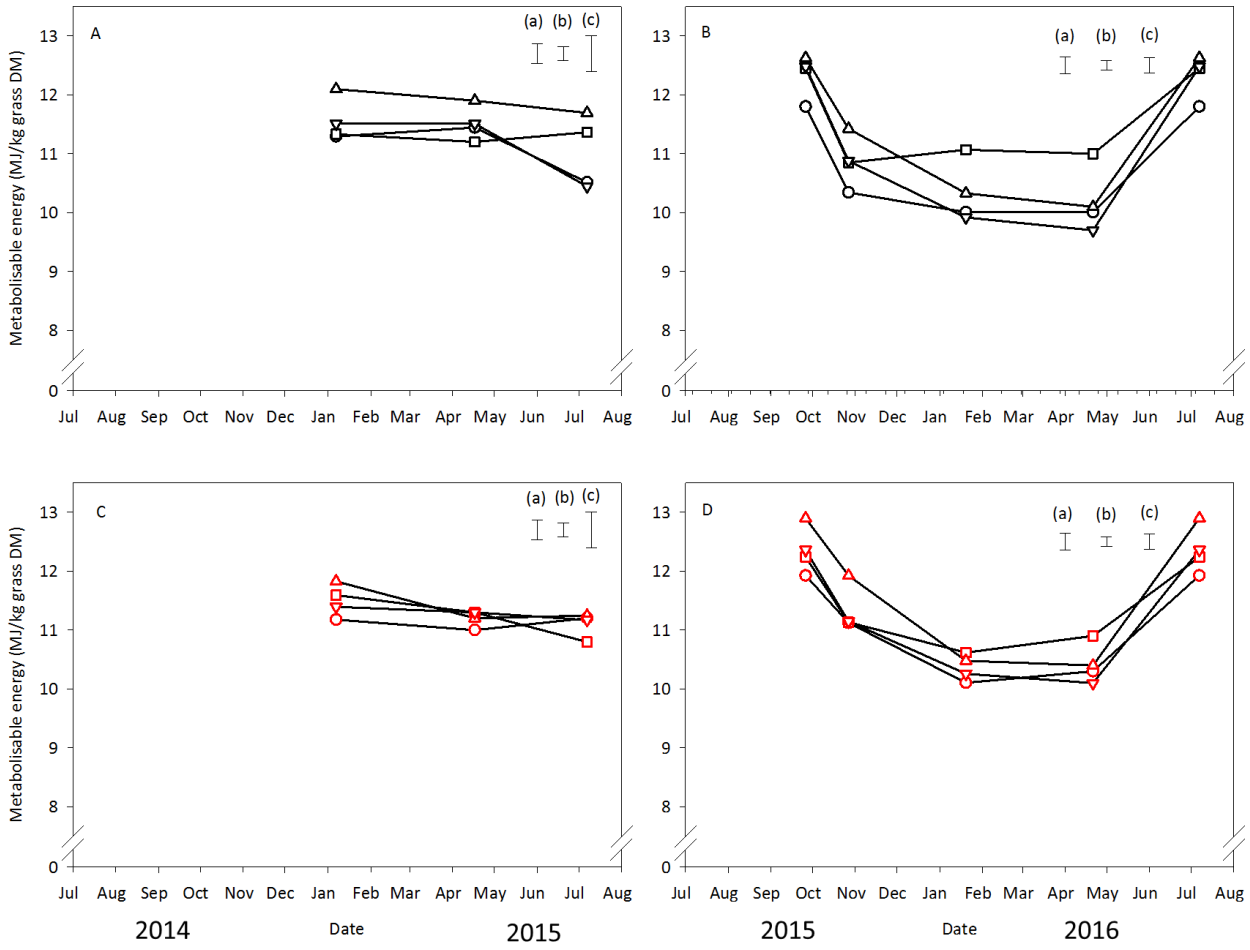


Figure 4-17 Metabolisable energy (ME) content (MJ/kg DM) for monocultures of +N (A and B) and −N (C and D) of brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) in 2014/15 and 2015/16 grown at Ashley Dene, Canterbury, New Zealand. Error bars are SEM for (a) species effects, (b) N effects and (c) species*N interactions of the total metabolisable energy.

4.11.11 Using thermal time to analyse seasonal temperature effect

The seasonal effects on pasture growth rates were explained by summarizing the impact of temperature using accumulated thermal time (Moot *et al.*, 2000) calculated with the base temperature of 3 °C and the optimum of 23 °C (Section 4.9.1).

4.11.12 Experiment 1 at Ladbrooks

First year (2014/15)

From 7/1/2015 to 9/6/2015 (before applying N fertiliser), the relationship between accumulated thermal time and accumulated DM was separated to three stages. In Phase 1 (between 7/1/2015 to 24/2/2015), the temperature adjusted growth rate increased ($P<0.001$) at an almost constant rate of 1.43 ± 0.15 kg DM °Cd^{-1} ha^{-1} above the base temperature for brome, tall fescue and perennial ryegrass

which was higher than 0.77 kg DM °Cd^{-1} ha^{-1} for cocksfoot (Figure 4-18). Phase 2 was between 1273 and 1699 °Cd for all species. Regressions were not fitted in Phase 2 since there were not enough destructive harvest data available in this phase. In Phase 3, the average temperature adjusted growth rate was 4.05 kg DM °Cd^{-1} ha^{-1} which was not different among species.

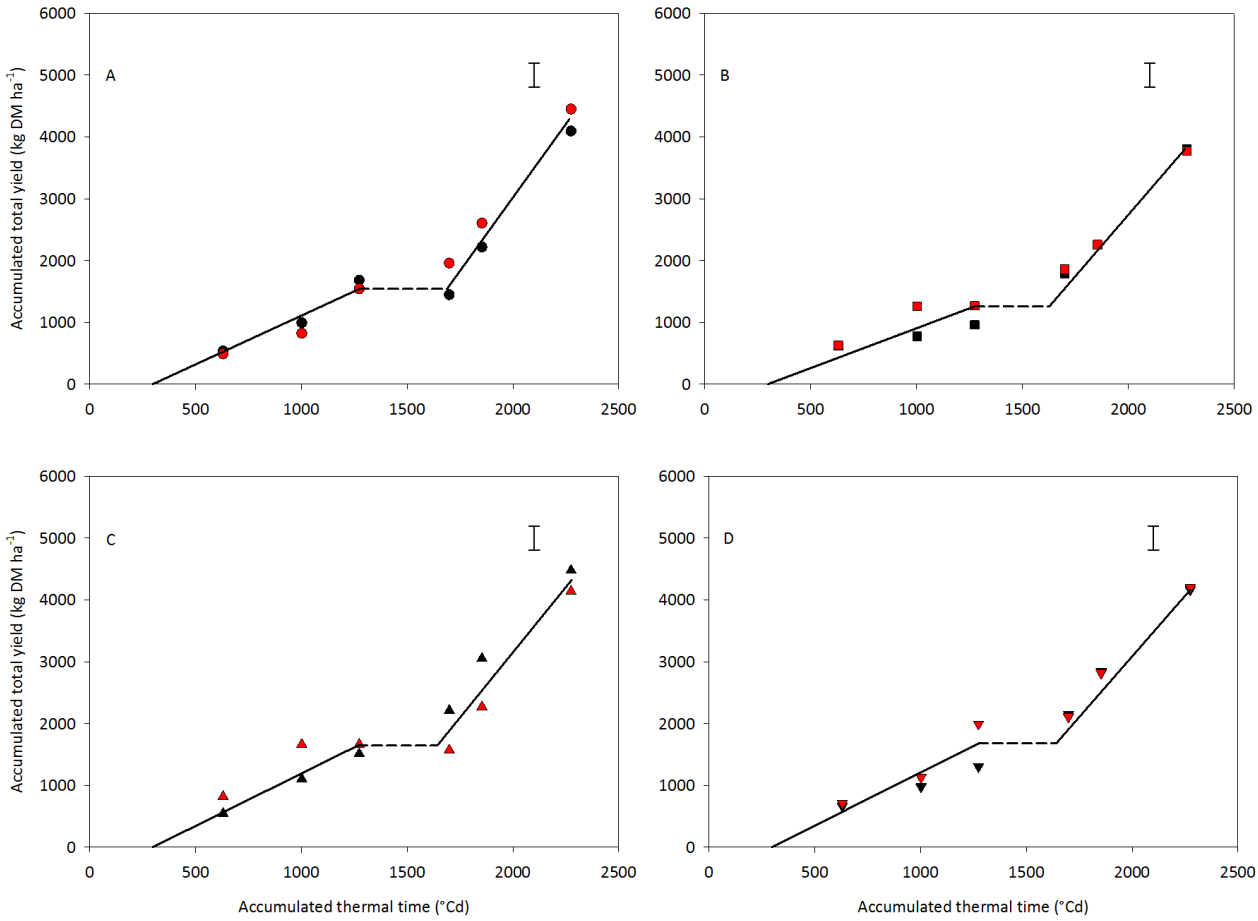


Figure 4-18 Dry matter (DM) accumulation by +N (black) and −N (red) of (A) brome (●,●), (B) cocksfoot (■,■), (C) perennial ryegrass (▲,▲) and (D) tall fescue (▼,▼) pastures (2014/15) against accumulated thermal time (Tt) with a base temperature of 3 °C at Ladbrooks, Canterbury New Zealand. Since there was no difference in total DM between + and −N treatments for all species, single regression lines fitted to the average of ±N data at each phase. Values are the average accumulated DM (destructive harvests) measured from 16/11/2014 (30 days after sowing) to 9/6/2015. Models were not fitted in Phase 2, but dashed lines (– – –) have been included to separate apparent phases based on the destructive harvest data. Error bars are SEMs of DM for species.

Second year (2015/16)

Figure 4-19 shows the linear regressions fitted to + and −N data and separated into two phases. Breaks indicate a reduced growth due to the onset of moisture stress (Chapter 5). The break points were not different (P=0.55) among species, but were later (P≤0.001) in +N compared with –N treatments. Phase 1 was from 10/6/2015 when accumulated thermal time was set at 0 °Cd for both ±N pastures. For +N pastures Phase 2 started from the middle of summer (almost on 6/3/2016), once accumulated thermal time was 2355 ± 44.6 °Cd. For −N treatments Phase 2 started earlier (P≤0.001), once accumulated thermal time was 2161 °Cd (almost on 19/2/2016).

In Phase 1, temperature adjusted growth rate was different among species (P≤0.001) and N treatments (P≤0.001). The mean temperature adjusted growth rate for cocksfoot and perennial ryegrass was 6.94 kg DM °Cd^{-1} ha^{-1} which was higher than brome and tall fescue (6.00 kg DM °Cd^{-1} ha^{-1}). There was no interaction (P=0.149) between species and N. Mean temperature adjusted growth rate for –N treatments was 4.38.

In Phase 2 temperature adjusted growth rate was also higher (P≤0.001) for +N treatment (Figure 4-19). In Phase 2, the temperature adjusted growth rate was between 1 to 1.43 kg DM °Cd^{-1} ha^{-1} for brome and perennial ryegrass which was higher (P≤0.05) than 0.50 kg DM °Cd^{-1} ha^{-1} for cocksfoot.

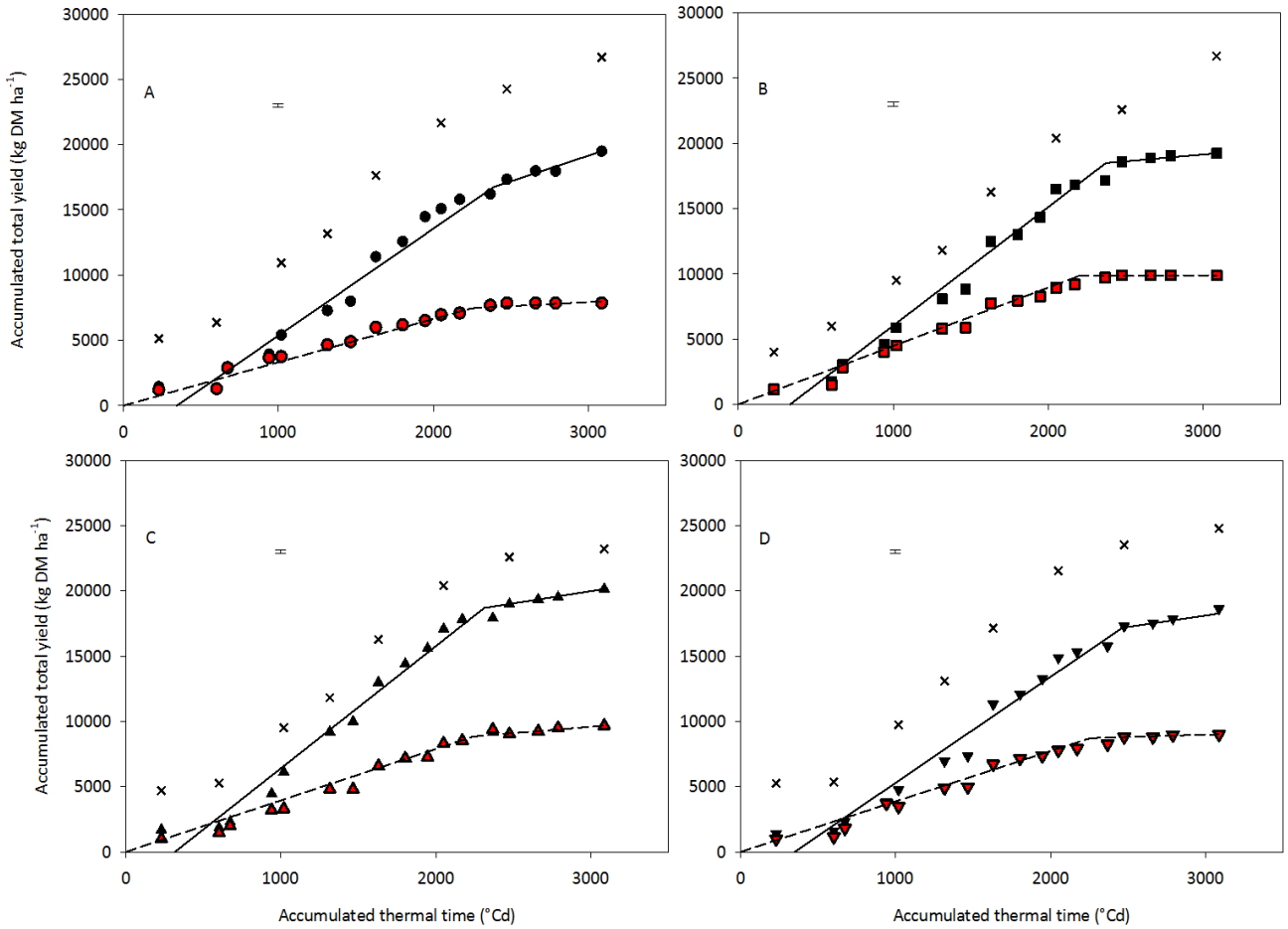


Figure 4-19 Dry matter (DM) accumulation by +N (black) and −N (red) of (A) brome (●,●), (B) cocksfoot (■,■), (C) perennial ryegrass (▲,▲) and (D) tall fescue (▼,▼) pastures (2015/16) against accumulated thermal time (Tt) with a base temperature of 3 °C at Ladbrooks, Canterbury New Zealand. Regressions fitted to destructive harvest data from +N (—) and −N (---) treatments. Values are the average accumulated DM measured from 10/6/2015 to 10/6/2016. Error bars are SEM for N. Arrows show the destructive harvests.

4.11.13 Experiment 2 at Ashley Dene

First year (2014/15)

In the establishment year at Ashley Dene (from 7/1/2016 to 7/7/2016), there was insufficient data to fit regression lines. Calculated temperature adjusted growth rate shown in Figure 4-20 (from 7/1/2015 to 17/4/2015) was 0.401 ± 0.032 kg DM °Cd^{-1} ha^{-1} and during winter (from 18/4/2015 to 7/7/2015) it increased to 3.03 ± 0.222 kg DM °Cd^{-1} ha^{-1}.

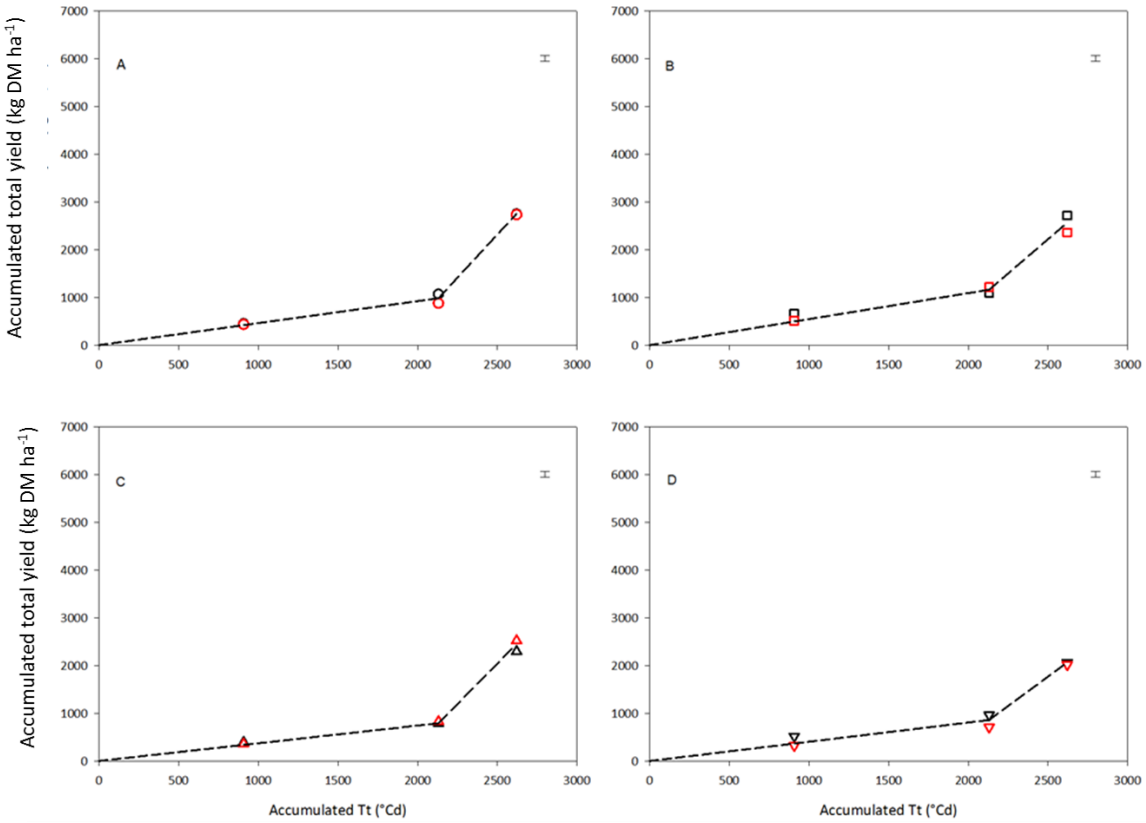


Figure 4-20 Dry matter (DM) accumulation by +N (black) and −N (red) for monocultures of (A) brome (○,○), (B) cocksfoot (□,□), (C) perennial ryegrass (△,△) and (D) tall fescue (▽,▽) pastures against accumulated thermal time (Tt) with a base temperature of 3°C in 2014/15 at Ashley Dene, Canterbury New Zealand. Values are the average accumulated DM measured from 16/10/2014 to 7/7/2015. Models were not fitted but dashed lines (– – –) have been included to separate apparent phases based on the destructive harvest data. Error bars are SEM for species.

Second year (2015/16)

Plotting DM data (destructive and non-destructive) against accumulated thermal time at Ashley Dene shows that in many cases, between two destructive harvests, pasture production has been limited (Figure 4-21). The linear regressions fitted to + and −N data at Ashley Dene separated to five phases. Breaks indicate increased/decreased growth due to the rainfall or onset of moisture stress (Chapter 5). Repeated measures analysis showed an interaction ($P<0.01$) between species and N in temperature adjusted growth rate in Phase 3.

Phase 1 was from 8/7/2015 when accumulated thermal time was set at 0 °Cd for both ±N pastures. For brome, cocksfoot and tall fescue, Phase 2 started from 12/10/2015, once accumulated thermal time was 500 °Cd. For perennial ryegrass, this started later, once accumulated Tt was 640 °Cd which was due to the higher DM production of ryegrass pastures measured on 27/10/2015 compared with brome and tall fescue. Phase 2, ended on 31/12/2015 for all pastures. In Phase 2, once accumulated rainfall was 85 mm, the average temperature adjusted growth rate for cocksfoot pastures was 1.19 kg

DM °Cd^{-1} ha^{-1} which was higher (P<0.001) than other grasses (0.03 kg DM °Cd^{-1} ha^{-1}). Phase 3 started on 31/12/2015, before 85 mm of rainfall in January. In Phase 3, N increased production of cocksfoot pasture. In this period, temperature adjusted growth rate for +N cocksfoot was 4.78 kg DM °Cd^{-1} ha^{-1} compared with an average of 3.24 kg DM °Cd^{-1} ha^{-1} for other species. DM production fluctuated among all species in both + and −N pastures in Phase 4, once accumulated thermal time was between ~1700 to 2600 °Cd (from 1/2/2016 to 11/4/2016). In Phase 5, once accumulated rainfall was 150 mm, temperature adjusted growth rate for cocksfoot was 2.08 kg DM °Cd^{-1} ha^{-1} which was higher (P<0.01) than the average of 0.822 kg DM °Cd^{-1} ha^{-1} for other species (Figure 4-21).

The mechanisms which caused yield differences during these phases at Ashley Dene, as investigated in Chapters 5 and 6.

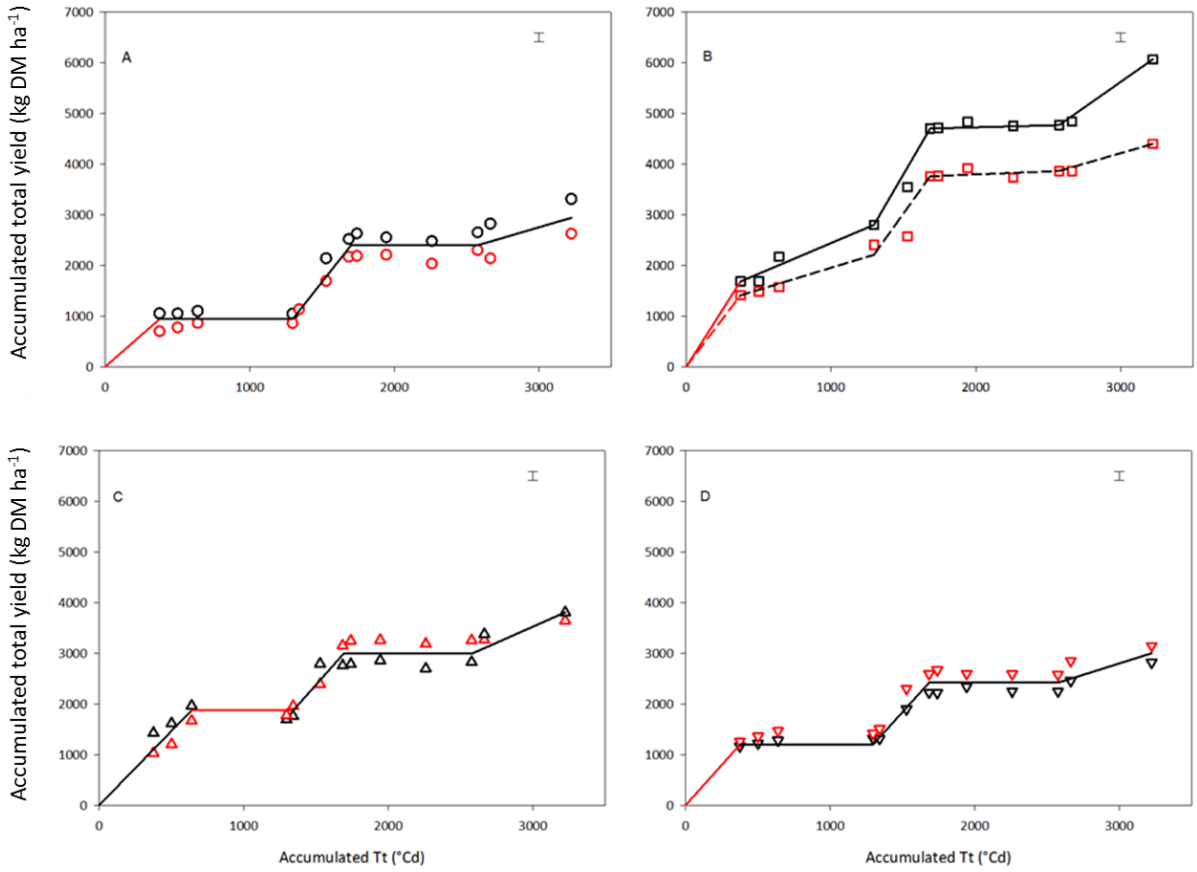


Figure 4-21 Dry matter (DM) accumulation by +N (black) and −N (red) for monocultures of (A) brome (○,○), (B) cocksfoot (□,□), (C) perennial ryegrass (△,△) and (D) tall fescue (▽,▽) pastures against accumulated thermal time (Tt) with a base temperature of 3 °C in 2015/16 at Ashley Dene, Canterbury New Zealand. Regressions from +N (—) and −N (----) treatments fitted to both destructive and non-destructive harvest data. Red lines (—) show when there was not enough data points to fit the regression lines. Since there was no difference in total DM between + and −N treatments for brome, perennial ryegrass and tall fescue, single regression lines fitted to the average of ±N data at each phase. Error bars are SEM for N.

The cause of difference in DM production between two sites is related to the different soil types which affect the amount of water available for growth (Section 4.5). The combined effects of N and plant available moisture needs to be quantified to further explain crop yield differences among ±N species, and between the two sites (Objective 5, Section 1.4).

4.12 Discussion

Objective 2 of this study was to generate dryland brome, cocksfoot, perennial ryegrass and tall fescue pastures with contrasting yield potential by using two sites which differed in PAWC. This was necessary to understand the effect of moisture on the production of monocultures of these grasses under dryland conditions. Then, Objective 3 was to define the combined effects of moisture and N on seasonal and annual dry matter production and pasture quality. Because establishment might affect dryland pasture production, Section 4.12.1 compares seedling establishment. Then in Section 4.12.2, differences in pasture production in the first year of study and subsequent year (2015/16) are discussed.

Objective 4 of this research was to quantify the DM production of each ±N species in relation to accumulated thermal time and soil moisture availability at Ladbrooks and Ashley Dene. The results are discussed in Section 4.12.2.

4.12.1 Pasture establishment

Two months after sowing, there was an indication (P=0.067) of a higher mean number of seedlings for all species at Ladbrooks compared with Ashley Dene. The number of seedlings/metre of drill row for perennial ryegrass was higher than brome and cocksfoot at Ladbrooks. At Ashley Dene, the number of seedlings for perennial ryegrass was ~1.5 times higher than other species. However, this was not significantly different (P=0.53) due to a high CV at Ashley Dene compared with Ladbrooks. The mean number of seedlings for tall fescue (63.4) was intermediate at both sites.

There was no interaction (P=0.64) among species in root weight between the sites. However, the mean root weight was lower (P<0.05) for cocksfoot than other species at both sites (Figure 4-5). The lowest seedling root biomass of cocksfoot at both sites is probably due to its slower germination/emergence (Chapter 3) and establishment (Charlton and Stewart, 1999) compared with the other grass species in the current study. Previous studies also showed that, cocksfoot germination/ emergence (McWilliam *et al*., 1970) and also establishment (Charlton and Stewart, 1999) were slower than brome, tall fescue and especially perennial ryegrass.

The lower PAWC at Ashley Dene compared with Ladbrooks caused 43.7%, 63.1% and 72.4% lower shoot biomass for brome, tall fescue and perennial ryegrass, compared with 28.9% for cocksfoot. These results demonstrate that cocksfoot was slower to establish. However, with the lower moisture content available in the Lismore stony soil at Ashley Dene, compared with the Wakanui silt loam soil at Ladbrooks, the cocksfoot seedlings root and shoot biomass were affected less than the other species.

After 62 DAS at Ladbrooks, shoot dry weight of perennial ryegrass, tall fescue and cocksfoot was 0.127, 0.101 and 0.0521 g which were similar to a previous study which showed 0.176, 0.91 and 0.34 g seedling weight respectively for the same species at 57 DAS (Moot *et al*., 2000). At the same time at Ashley Dene, shoot dry weight of perennial ryegrass and tall fescue was ~0.045 g which was four and two times less than the results from Ladbrooks and also the results showed by Moot *et al*. (2000). This also confirms the effect of drought on shoot weight of perennial ryegrass and tall fescue which influenced shoot weight of ryegrass seedlings more than tall fescue under the same level of moisture stress at Ashley Dene.

At both sites, seedling shoot dry weight was higher ($P<0.01$) for brome and perennial ryegrass. Compared with other species, the larger seeds for brome contain more seed reserve and appear to have enabled its seedlings to grow faster than other species. Shoot dry weight for perennial ryegrass seedlings was higher than tall fescue and cocksfoot which is reportedly be due to its use of endosperm reserve for faster germination/emergence (Brock *et al*., 1982). Earlier emergence also allowed plants the ability to expand the leaves, intercept light and utilise moisture and nutrients earlier faster than slower emerging species. The rapid emergence of perennial ryegrass (Section 2.8.6) is one of the reasons it is favoured as a pasture species even though it may not persist dryland environments.

The root/shoot ratio was not different among species but was higher at Ashley Dene, due to the high shoot dry weight ($P<0.01$) at Ladbrooks. This suggests, the higher PAWC at Ladbrooks did not affect root biomass but enabled higher shoot DM compared with Ashley Dene which was due to higher leaf expansion and radiation interception by the seedlings leaves at Ladbrooks. Reduced leaf area is the most obvious response of plants to restricted water availability (Section 2.3.1), and this caused seedlings to have less shoot weight at Ashley Dene with a lower PAWC compared with the seedlings at Ladbrooks.

Easton (1994) reported that the slower establishment of tall fescue particularly compared with ryegrass, is a limitation for its use, and affects its production in the establishment year. However, this was not the case in the current study. The hydrothermal time results (Chapter 3) showed that, ≥60% of 'Finesse Q' tall fescue seeds were able to germinate in a wide range of temperature and water limitation (Section 3.5.5). Also the field experiment results at Ashley Dene showed that, 62 days after sowing, 42% of the tall fescue sown seeds had emerged (Section 4.11.1). This was similar to the number of emerged perennial ryegrass seedlings and was higher than the number of cocksfoot and brome seedlings at the same time. From sowing date to 4/5/2015, before the second application of herbicide at Ashley Dene, tall fescue showed the highest proportion (23%) of the weeds (of mostly browntop and fathen reported in Section 4.11.6) compared with other grasses. This was comparable with 17% for cocksfoot. However, tall fescue production was more vulnerable to invasion by weeds than other

grasses at Ashley Dene. Previous studies also reported that tall fescue is more affected to invasion by weeds such as browntop if fertility requirements are limited, like the situation at Ashley Dene in this study (Lambert *et al*., 2004, Saul and Chapman, 2002).

The number of tillers per plant was not measured in the current study. However, field observations showed that tall fescue had relatively fewer tillers per plant, once established. This is consistent with previous studies that showed, new tiller formation in tall fescue is one-third slower than ryegrass (Milne *et al*., 1997).

4.12.2 Dry matter yield and pasture growth

Before applying N, and in the establishment year (from 17/10/2014 to 18/9/2015 at Ladbrooks and from 16/10/2014 to 21/9/2015 at Ashley Dene), total DM of all grasses was not different among species at Ladbrooks (Figure 4-6) or Ashley Dene (Figure 4-7). The average total DM at Ladbrooks was 4.34 ± 0.330 t ha^{-1} which was more than two times higher than the production at Ashley Dene (1.93 ± 0.116 t ha^{-1}). The difference in total DM produced by pastures in 2014/15 (also 2015/16) shows that Objective 2, to generate swards with different annual dry matter potential through the two sites which differ in PAWC was achieved. The causes of the yield differences will be explained in detail in Chapters 5 and Chapter 6.

Objective 3 was to describe the effects of these different moisture regimes (at Ladbrooks and Ashley Dene) and N levels on seasonal and annual DM production and the quality of dryland brome, cocksfoot, perennial ryegrass and tall fescue pastures in the establishment (2014/15) and subsequent year (2015/16). Pasture production is also related to the establishment of the pastures at each site. Therefore, this would be discussed as a factor which affects production of the pastures at the both sites.

At 84 days after sowing in January 2015, daily growth rate was similar and lowest at the both sites (Figures 4-8 and 4-9 A and C). However, once grasses established at Ladbrooks (February and March 2015), daily growth rate increased to 31.5 kg DM ha^{-1} d^{-1}. Mean daily growth rate at Ashley Dene, was 5-8 kg DM/ha/d from January to July 2015. The low daily growth rate at Ashley Dene was expected due to the Lismore stony silt loam soil and its lower moisture availability compared with a Wakanui silt loam soil at Ladbrooks (Section 4.5). At both sites, DM of brome was higher than other species in the winter (June 2015 at Ladbrooks and July 2015 at Ashley Dene) as it's a winter active grass species (Stewart, 1996b). This was consistent with reports in cold climates such as Lees Valley in North Canterbury. For example, once temperature decreased to 9.3 °C 'Bareno' brome showed the highest ($P<0.01$) yield (1.10 t DM ha^{-1}) among 10 grass monocultures in a rotation between 12/4/2008 to 5/6/2008 (Fasi *et al*., 2008).

In 2014/15, before herbicide application at Ashley Dene, the proportion of weeds in total DM of cocksfoot (and tall fescue) was higher ($P<0.05$) than perennial ryegrass and brome (Figure 4-10). Also, before the second herbicide application at Ladbrooks, cocksfoot had the highest ($P<0.05$) proportion of weeds in May 2015 which caused a reduction ($P\leq0.05$) in cocksfoot yield due to competition. This can also be attributed to the slower germination of cocksfoot (Charlton *et al*., 1986). Hay and Ryan (1983) reported that yields of weeds were greater in the plots that contained slower establishing species including 'K1858' cocksfoot. The slower establishment of cocksfoot required weed control at establishment. However, once it is established, its growth habit makes weed content of older cocksfoot to be usually lower. For example, Mills (2007) reported that, nine years after establishment, total dry matter production of cocksfoot pastures contained only 6% weeds.

In the establishment year (2014/15) at Ladbrooks, DM production of tall fescue was 4.31 t DM ha^{-1} yr^{-1} and similar to the other species. Production of tall fescue in the establishment year at Ladbrooks was lower than the yields for 'Advance' (7.8 t DM ha^{-1} yr^{-1}) and 'Flecha' (6.90 t DM ha^{-1} yr^{-1}) in their establishment year (2008) in a Templeton silt loam soil at Lincoln University, Lincoln, Canterbury, New Zealand (Jusoh, 2013). Tall fescue has previously been reported as a drought tolerant species (Section 2.8.2). However, in the same year (2014/15) and under more severe moisture stress at Ashley Dene, there was no advantage to tall fescue over brome and perennial ryegrass. The inability of tall fescue to produce higher DM compared with the other species at Ashley Dene can be related to its poor performance in the establishment year as shown by its vulnerability to weed invasion and also lower tiller production (Section 4.12.1.) which probably reduced light interception until canopy closure, relative to the other species.

In the second year (from 8/7/2015 to 7/7/2016) at Ladbrooks, annual yield in +N pastures was 19.8 ± 0.44 t DM ha^{-1} yr^{-1} (Figure 4-6) compared with the potential of 21.7 t DM ha^{-1} yr^{-1} estimated for dryland perennial ryegrass pastures in Canterbury (Mitchell, 1963). Total yield of +N pastures at Ladbrooks was higher than the 15.1 t DM ha^{-1} yr^{-1} for the dryland +N 'Wana' cocksfoot reported by Mills *et al*. (2007) on a Templeton silt loam soil and the 11.5 t DM ha^{-1} yr^{-1} from dryland +N 'Grasslands K1858' cocksfoot sown on a Wakanui silt loam soil (Vartha, 1977) at Lincoln. DM of +N cocksfoot at Ladbrooks was 70% of the environmental potential (28.6 t DM ha^{-1}) reported by Stevens *et al*. (1992) in Canterbury. In 2015/16 at Ladbrooks, pastures that received no N yielded <50% of the +N pastures. The potential to double yield in the second year was consistent (Figure 4-6) for all species. As temperature increased in summer 2016 (January/February), the difference in DM production between + and −N treatments became greatest at Ladbrooks. At this time the mean daily growth rate of all species were almost three times higher in +N treatments (160 kg DM ha^{-1} d^{-1}) compared with −N treatments (Figure 4-8). This supports previous studies by Peri *et al*. (2002) for 'Grasslands Wana' cocksfoot which showed the

maximum difference in DM production between higher N urine patches and controls (380 kg DM ha^{-1} d^{-1}) occurred in summer. At Ladbrooks, daily growth rate of the ±N pastures decreased in March and April 2016, when temperature was still non-limiting for plants growth. The reductions in daily growth rates was expected due to moisture stress (Chapter 5).

In the second year (from 8/7/2015 to 7/7/2016) at Ladbrooks, DM production of all −N species was not different (9.21 t DM ha^{-1} yr^{-1}). −N cocksfoot yield was higher than yields consistently reported for cocksfoot pastures (5 t DM ha^{-1} yr^{-1}) in dryland Canterbury and were 42% of the environmental potential for cocksfoot in Canterbury (Stevens *et al*., 1992). Cocksfoot control yield (−N cocksfoot) was similar to those reported by Peri (2005) under grazing (9.2 t DM ha^{-1} yr^{-1}) but higher than the 7.6 t DM ha^{-1} yr^{-1} reported by Stevens *et al*. (1992) and others. DM production of the pastures in 2015/16 at Ladbrooks showed that brome, cocksfoot and tall fescue, which are reported as drought tolerant species suited to low-moderate fertility conditions (Section 2.8) were all capable of high yields in dryland pastures with a moderate moisture deficit and a relatively high PAWC when N was applied. In the same year (2015/16), and under an extreme moisture stress at Ashley Dene, DM of +N cocksfoot showed a positive response to 85 mm of rainfall more than the other species did. When N was not limited, increasing temperature in the summer (from January-May 2016) caused an increase in mean daily growth rate of cocksfoot more than other species. Previous studies showed that in dryland Canterbury, N rather than moisture was the factor most limiting cocksfoot production (Peri *et al*., 2002; Mills *et al*., 2006). At the same time, brome, perennial ryegrass and tall fescue did not respond to the summer N application.

Tall fescue has previously been reported to respond to irrigation (Lowe and Bowdler, 1995). However, the 85 mm of rainfall at Ashley Dene in January 2016 (Figure 4-7) was insufficient to induce a response with only ~1 t DM ha^{-1} increase in production in both + and −N treatments (Section 4.11.4). The results of the current study are consistent with previous studies which showed that, the difference in annual production occurred because cocksfoot produced 131% higher DM than ryegrass in summer and 74% higher DM in autumn at Lincoln, Canterbury, New Zealand. Radcliffe and Baars (1987) also demonstrated a higher rate of growth for cocksfoot (65 kg DM ha^{-1} d^{-1}) compared with ryegrass (35 kg DM ha^{-1} d^{-1}) in the summer. Maximum growth rate generally happens later than in ryegrass (Radcliffe and Baars, 1987). The main cause was the different optimum temperature of different species for photosynthesis (Peri, 2002). Higher daily growth rate of +N cocksfoot compared with the other grasses after January rainfall in 2016 (Section 4.11.5) could be a consequence of cell expansion of leaves initiated under moisture stress as cell expansion is the first process affected by moisture stress but cell division can continue even if there is insufficient moisture available for expansion (Hsiao, 1973). In this

case there was a benefit to cocksfoot, where production over other grasses in summer and autumn, which is consistent with previous reports from Radcliffe and Baars (1987).

From July to December 2015 (before the rainfall event in January 2016) dry matter production of all grasses was similar in + and −N treatments (Figure 4-7). From 27/2/2016 to 27/3/2016, when accumulated rainfall was <30 mm at Ashley Dene, only cocksfoot showed a positive response to N. However, non-destructive data show that the increase in DM production of cocksfoot at this time was due to the accumulation of DM for cocksfoot between 15/1/2016 to 27/2/2016, once moisture was still available from the 85 mm rainfall event in January. DM production of all ±N pastures levelled off from 27/1/2016 to 27/3/2016 at Ashley Dene. This confirms that, for N uptake from the soil, moisture is required. Once available moisture was insufficient, even when N was not a limitation to growth, production only proceeded at the rate allowed by the most limiting factor (moisture).

Effective usage of applied N fertiliser by the crop will decrease input cost per unit of product harvested. The N recovery results in this research show that, under severe moisture stress at Ashley Dene, N recovery by cocksfoot was almost two to three times higher than other +N pastures. Comparing total N recovery by pastures before application of N in the first year of study at Ashley Dene showed that, total N recovery by cocksfoot and brome was higher than other grasses. However, at the same time at Ladbrooks there was no difference among species in total N recovery. In 2015/16 at Ladbrooks, almost half of a total application of 900 kg N ha^{-1} was recovered by the pastures and this was not different among species. This is consistent with the previous studies reporting that half of the applied N fertiliser can be expected to be recovered by the crops (Fenn and Hossner, 1985). The rest of applied N can leach below the root zone with water, nitrate can be converted to gaseous forms in anaerobic environments, and NH3 can be lost to the atmosphere. However, the intensity of loss from each mechanism is related to the soil water content and texture, temperature and form of N, and other environmental factors (Fenn and Hossner, 1985). As an example, under a severe drought conditions at Ashley Dene, application of N (500 kg N ha^{-1}) only increased N recovery by brome, cocksfoot and perennial ryegrass. Since the current study was under dryland conditions, it is less likely that the amount of N which was not recovered by the plants has been leached below the root zone with water. The lost N at Ashley Dene might be lost as the form of NH_3 to the atmosphere. Since at Ashley Dene, the only occasion in which, N improved pasture production was after 85 mm of rainfall in January 2016 for cocksfoot, application of N does not recommended for the other grasses used in this study under a severe moisture stress. Under this condition, application of N is no cost effective and environmentally friendly.

In 2015/16, +N pastures at Ladbrooks had a minimum of 3.5 times (for cocksfoot) and a maximum of 6.65 times (for tall fescue) more annual crude protein at Ladbrooks than Ashley Dene. Annual crude

protein of −N pastures at Ladbrooks was 1.68 (for tall fescue) to 3 (for brome) times more than Ashley Dene. Total crude protein in the second year at Ladbrooks was 3-4 times more in +N compared with −N treatments. The results show that, in 2015/16 at Ladbrooks, the main cause of differences in pasture quality within the sites was N.

Annual CP production was not different among species. At Ladbrooks, total CP for all species was between 3.9 to 4.2 (t ha^{-1}) which is similar to dryland +N 'Wana' cocksfoot in 2004/5 (3.9 t ha^{-1}) and comparable with those reported for lucerne (4.6-4.4 t CP $ha^{-1}yr^{-1}$) by Brown (2004). In 2015/16 at Ladbrooks, the main cause of differences in pasture quality was N. Grass species, in this experiment achieved a similar annual CP production to lucerne at a similar site reported by Brown, (2004) which was due to application of N (800 kg ha^{-1} yr^{-1}) in 2015/16. The lucerne received no N and produced its CP yields by biological N fixation, and the extraction and use of available soil N and utilisation of N returns from grazing livestock. Nitrogen fixation by legumes is important in the success of pastoral agriculture in New Zealand. Application of 800 kg N ha^{-1} yr^{-1} used in this study is not recommended for commercial use. Peoples and Baldock (2001) reported that 25 kg N was fixed per each tone of legume DM. Thus, maintenance of a high proportion of legume in the pasture will improve pasture quality. In 2015/16, −N pastures annual CP yields was between 1 to 1.3 t CP ha^{-1} yr^{-1} since they were completely reliant on the available soil N. The annual CP yields in −N treatments at Ladbrooks are higher than 0.6 t CP ha^{-1} yr^{-1} which was previously reported for −N dryland 'Wana' cocksfoot and is similar to irrigated −N 'Wana' cocksfoot (1.2 t CP ha^{-1} yr^{-1}) reported by Mills (2006) on a Templeton silt loam soil. Both in + and −N treatments, herbage quality in terms of ME and CP was lowest for all species in early summer. Lower pasture ME in early summer compared with autumn and winter (Figures 4-16 and 4-17) was expected. The reasons for seasonal variations in pasture ME were reviewed in Section 2.6.2.

Objective 4 was to quantify the DM production of each ±N species in relation to accumulated thermal time and soil moisture availability. The mechanisms which caused yield reduction under two moisture levels at Ladbrooks and Ashley Dene are investigated in Chapter 6.

The average temperature adjusted growth rate of +N pastures at Ladbrooks, was ~6-7 kg DM °Cd^{-1} ha^{-1} until the middle of summer (from 0 to 2355 ± 44.6 °Cd). This was not different among species and was almost 80% higher than the average temperature adjusted growth rate for −N pastures at the same time. The temperature adjusted growth rate by +N pastures was similar to the 7.2 kg DM °Cd^{-1} ha^{-1} reported by Mills (2006) for irrigated +N and 7 kg DM °Cd^{-1} ha^{-1} for dryland +N 'Wana' cocksfoot once the period of moisture stress was excluded in Canterbury.

With the onset of moisture stress in Phase 2 at Ladbrooks, temperature adjusted growth rate was not different between + and −N treatments. In contrast, at Ashley Dene, the linear regressions fitted to + and −N data separated to five phases. The results both at Ladbrooks and Ashley Dene show that when

available moisture was insufficient for N uptake, in both + and −N pastures, production only proceeded at the rate allowed by the most limiting factor (moisture). Plants inability to uptake N with a lower PAWC and prolonged moisture stress at Ashley Dene occurred in four phases. The results at Ashley Dene indicate that, the minimum soil moisture for N uptake was lower for cocksfoot compared with the other grasses used in this study. This resulted in a positive response to N for +N cocksfoot which caused a 1.5 kg DM °Cd^{-1} ha^{-1} higher production after 85 mm of rainfall in Phase 3 compared with the other species.

At Ashley Dene, Phase 2 started later for perennial ryegrass. This was mainly due to higher production of perennial ryegrass in spring which resulted in a higher DM production of ryegrass compared with tall fescue and brome between 27/10/2015 and 15/11/2015. Therefore at Ashley Dene, only on one occasion was perennial ryegrass production higher than that of brome and tall fescue, but similar to cocksfoot which caused an additional 140 °Cd of growth for ryegrass in Phase 1.

The annual yield variations between sites (Ladbrooks and Ashley Dene) show the impact that soil type caused. The timing, extent and duration of the drought period ultimately determine the production reduction in dryland pasture (Jamieson, 1999). Therefore, in Chapters 5 and 6 mechanisms affected the production of ±N pastures are investigated.

4.13 Conclusions

The results in this chapter showed that, Objective 2 which was to generate and grow pastures with contrasting yields was achieved. Then the combined effects of moisture and N regimes on seasonal and annual dry matter production and pasture quality were quantified (Objective 3).

Based on these results the following conclusions can be drawn:

- In a Wakanui silt loam soil at Ladbrooks, with a higher PAWC compared with Ashley Dene, there was no difference in DM production among species. The main factor that affected species production was N, as DM was almost two times greater in +N compared with −N treatments.
- In a Lismore stony silt loam soil at Ashley Dene, when N was not limited in the established pastures in 2015/16, cocksfoot production was 46-60% higher than perennial ryegrass, tall fescue and brome. Over the five regrowth cycles at Ashley Dene, cocksfoot showed higher DM on three occasions compared with the other species. This was achieved through a higher daily growth rate in summer at Ashley Dene. On only one occasion at Ashley Dene there was an interaction between species and N. Cocksfoot showed a positive response to N once pastures received 85 mm of rainfall in January 2016. Increasing DM production of +N cocksfoot was due to the ability of the plant to survive and recover quicker after drought.

- At Ladbrooks, N was the main factor affecting total but not absolute ME. Application of N in 2015/16 caused a 100-150% increase in total ME of all species. ME for brome was lower and for perennial ryegrass higher than other species in both 2014/15 and 2015/16.

- In 2014/15, total N recovery at Ladbrooks was not different among species. Total N recovery was 435 ± 17.17 kg N ha^{-1} which was higher in +N pastures but not different among species in 2015/16. Total CP in +N pastures at Ladbrooks was 3.42 ± 0.17 which was 150-200% higher than total CP in −N pastures. In 2015/16, annual CP was between 3.9-4.1 t CP/ha in +N treatments compared with 1-1.3 t CP/ha in −N treatments.

- At Ashley Dene, application of N did not affect total ME for perennial ryegrass and tall fescue. However, annual ME of both perennial ryegrass and cocksfoot was higher than other species in 2015/16. Nitrogen caused a 38% increase in total CP for cocksfoot and 42% in brome compared with −N treatments.

- In 2014/15, before application of N, total CP yield was higher ($P<0.01$) for brome and cocksfoot. Total N recovery as well as total CP by cocksfoot was higher than other species in 2015/16.

Objective 4 which was to quantify the production of each ±N species in relation to accumulated thermal time under different soil moisture availability was also achieved.

- The effect of temperature on seasonal pastures growth separated in two phases in 2015/16 at Ladbrooks. Under less limited moisture stress, growth rates were greatly driven by the variations in seasonal temperature. In Phase 1, before the onset of moisture stress in summer, +N pastures produced an average of 6-7 kg DM °Cd^{-1} ha^{-1} compared with 4.38 DM °Cd^{-1} ha^{-1} for −N pastures. In Phase 2, brome and perennial ryegrass pastures produced an average of 1.40 kg DM °Cd^{-1} ha^{-1} which was higher than 0.50 kg DM °Cd^{-1} ha^{-1} for cocksfoot.

- In 2015/16, the effect of temperature on seasonal pastures growth at Ashley Dene was separated in five phases. In three phases, cocksfoot pastures showed a higher response to temperature. In one phase, after 85 mm of rainfall in January, there was an interaction between temperature adjusted growth rate and species showing that only cocksfoot responded to N in that time.

In the following chapter, the relation between pasture production and PAWC at each site will be identified. Then, the combined effects of different levels of N and moisture on pastures water use and water use efficiency will be described.

Chapter 5 DM production and soil water use by dryland pastures

5.1 Introduction

Differences in DM production of brome, cocksfoot, perennial ryegrass and tall fescue under different soil types and N availability (Section 4.11.4) showed that objective to generate swards with diverse annual dry matter potential (Objective 2) was achieved. Objective 5 of this study (Section 1.4), is to quantify the PAWC of the two soil types.

To account for the seasonal impact of temperature on yield, DM production of ±N pastures was described in relation to thermal time at the both sites (Section 4.11.11). At Ladbrooks, there were consistent linear relationships between accumulated thermal time and DM production in both ±N pastures from 10/6/2015 when accumulated thermal time was set at 0 °Cd to the middle of summer. However, the linearity ceased in the middle of summer. Therefore, the relationship between accumulated thermal time and DM was separated to two phases at Ladbrooks. At Ashley Dene, five phases were required to describe the relationship (Section 4.11.11). The expectation is that this occurred because of moisture stress and this analysis examines this. Therefore, Objective 6 (Section 1.4) is to describe changes in actual soil moisture deficit at the two sites over the duration of the study in response to the sown pasture species.

As it was discussed in Section 2.1, yield formation is a function of PAR intercepted by the canopy and the radiation use efficiency (Equation 2-1). However, canopy expansion might be restricted by moisture stress which causes a reduction in the amount of light intercepted by the crop/pasture (Hsiao, 1973; Belaygue *et al*., 1996). As it was shown in Chapter 4, under the same level of moisture stress at Ashley Dene, DM production of +N cocksfoot was almost two times more than the other species used in this study. The superior recovery of +N cocksfoot compared with the other species subjected to moisture stress may reflect differences between the species in the ability to access and extract soil moisture and/or water use efficiency. Therefore, Objective 7 is to calculate water use and water use efficiency of dryland pastures under different levels of moisture and N.

5.2 Materials and Methods

Details of experimental design and treatments (Section 4.7.1), soil tests results (Section 4.7.4), weed management (Section 4.7.5), mowing and grazing (Section 4.7.6) and N fertiliser application (Section 4.7.7) were presented in Chapter 4. Briefly, soil water measurements were made on 13 dates at Ladbrooks and 10 dates at Ashley Dene in all plots. This section describes methods used to measure

the effects of different levels of moisture on DM yield. The principles and relevant literature on water use and extraction were explained in Chapter 2.

5.2.1 Soil water content (SWC)

Soil water content was measured with a neutron probe. This required installation of aluminium access tubes at the centre of each plot (Plate 5-1) at the both sites.

Plate 5-1 Ladbrooks experimental site on 26/4/2016. The neutron probe access tubes are located towards the centre of each plot.

Access tubes were installed 10 days following the sowing dates, to allow mechanical sowing of the entire plot but minimise any damage to emerging seedlings.

A single 50 mm access hole was augured in each plot at Ladbrooks. Using this conventional installation method was not possible for the compacted gravels throughout the soil profile at Ashley Dene. Therefore, a 50 mm steel spike and a vibrating head attachment on a 20 tonne excavator were used to create the access holes at Ashley Dene. The aluminium tubes (47 mm outside diameter) were then

installed to a depth of 2.5 m at Ladbrooks and 2.0 m at Ashley Dene. Previous studies in similar soil types using the same installation methods did not affect actual soil moisture (Mills, 2007; Sim, 2014). Due to equipment failure in the first year, measurements of soil moisture are only presented from 17/4/2015 to 7/7/2015 at Ashley Dene. Due to the same reason, at Ladbrooks also the results of four out of seven measurements were reported from 29/1/2015 to 20/4/2015, in the first year.
In this chapter, soil moisture measurement data are presented from 15/10/2015 to 20/4/2016 in the second year of study at Ladbrooks. At Ashley Dene data are reported from 8/7/2015 to 21/4/2016 in the second year of study. Therefore, the main period of soil moisture deficit in summer (December-February) of the second year was quantified at both sites.

5.2.2 Volumetric soil water content (VWC)

To measure VWC of the top layer (0-0.2 m) a portable time domain reflectometer (TDR; Trace system, Soil Moisture Equipment, Santa Barbara, California, USA) was used within 0.1 m of the neutron probe access tube at both sites.

From the soil depths of 0.2 to 2.5 m at Ladbrooks and 0.2 to 2 m at Ashley Dene, neutron probe (Troxler Electronic Industries Inc, Research Triangle Park, North Carolina, USA) readings of soil moisture were taken at each 0.1 m depth increments. From 1/10/2015 to 20/4/2016 in the second year, soil water content was measured by using a neutron probe (CPN, 503 DR Hydroprobe, CPN International, CA, USA). The switch between instruments was required because of failure in the Troxler which was sent for repair. Thus, to ensure the accuracy of CPN, on two occasions, CPN and Troxler neutron probes were cross calibrated in four plots (Appendix J).

5.2.3 Soil moisture content (SMC)

Soil moisture content (mm) for the profile was calculated by using Equation 5-1.

Equation 5-1 $SMC = \sum_{bot}^{top} \Theta * \mathrm{d}$

In this equation Θ is the measured volumetric water content (mm^3/mm^3) of each soil layer in the profile and it is summed to the deepest measurement depth (d (m)).

5.2.4 Drained Upper Limit (DUL) and lower limits (LL) to plant water extraction

The drained upper limit (mm) was defined as the maximum stable volumetric water content. To do this, measurements were recorded at least five days after a rainfall event of >100 mm. To estimate DUL at both sites, a check value was obtained in the next year (on 9/5/2017) after 216 mm of rain fell over 66 days. This was expected to have fully recharged both soil profiles. For each measurement date,

the actual soil moisture deficit was then defined as the difference between the soil moisture content on the date and DUL for each individual 0.1 m of soil layer. The difference was then accumulated for 0-2.5 m at Ladbrooks and 0-2.0 m at Ashley Dene and reported as the actual soil moisture deficit.

At each site, the actual soil moisture deficit increased during known periods of moisture stress. The lower limit (mm) to plant water extraction was identified as the mean of the two lowest measured VWC within individual soil layers.

5.2.5 Plant available water content (PAWC)

The difference between DUL (mm) and LL (mm) was used to define the PAWC (mm) in each 0.1 m soil layer of dryland pastures and calculated based on Equation 5-2.

Equation 5-2 $PAWC = \sum (DUL - LL) * \ maximum\ extraction\ depth\ (m)$

5.2.6 Water use (WU)

The amount of water use (mm) in the period between two measurements was calculated for each plot using Equation 5-3.

Equation 5-3 $WU = P_{(R)} - (SMC_i - SMC_t)$

In Equation 5-3, $P_{(R)}$ is the sum of precipitation (mm) from rainfall over the measurement period, SMC_i is the SMC (mm) on the current day and SMC_t is the soil moisture content (SMC) at the earlier measurement. These were summed to calculate annual and regrowth cycle water use.

Previous studies (French and Legg, 1979; Brown *et al.*, 2012) showed that crop demand can be anticipated using atmospheric data, through Penman evapotranspiration. In the current study, on some occasions (8/11/2015, 8/12/2015 and 7/1/2016) there was a gap (4 to 14 days) between the herbage harvest dates and when that soil moisture was measured. Therefore, to predict crop water use, accumulated daily Penman potential evapotranspiration (EP) plus accumulated daily rainfall were considered as the quantity of water use in those three occasions of 4 to 14 days gaps between the harvest dates and measurement dates.

5.2.7 Water use efficiency (WUE)

Water use efficiency (kg DM ha^{-1} mm^{-1}) is defined as the slope of the linear regression of the relationship between accumulated DM yield (kg ha^{-1}) and cumulative water use (mm) calculated from Equation 5-3. Linear regressions were fitted to annual DM production against cumulative water use for individual plots.

5.2.8 Statistics

To compare different species and N treatments at each site, soil moisture deficit, water use and water use efficiency were analysed using a strip-plot design with ±N as the rows and pasture species as the columns. The variations in water use within individual regrowth cycles are caused by the environmental variables, solar radiation, PET, temperature and VPD (French and Legg, 1979). Any difference in water use in each regrowth cycle was analysed using repeated measures.

5.3 Results

5.3.1 Plant available water content of the soil (PAWC)

Experiment 1 at Ladbrooks

From 15/10/2015 to 20/4/2016, the estimated PAWC was not different (P=0.521) among species. This was 254± 7.51 mm (0-1.56 ± 0.629 m) for +N pastures which was higher (P<0.001) than the 195 mm (0-1.45 m) for −N pastures (Figure 5-1).

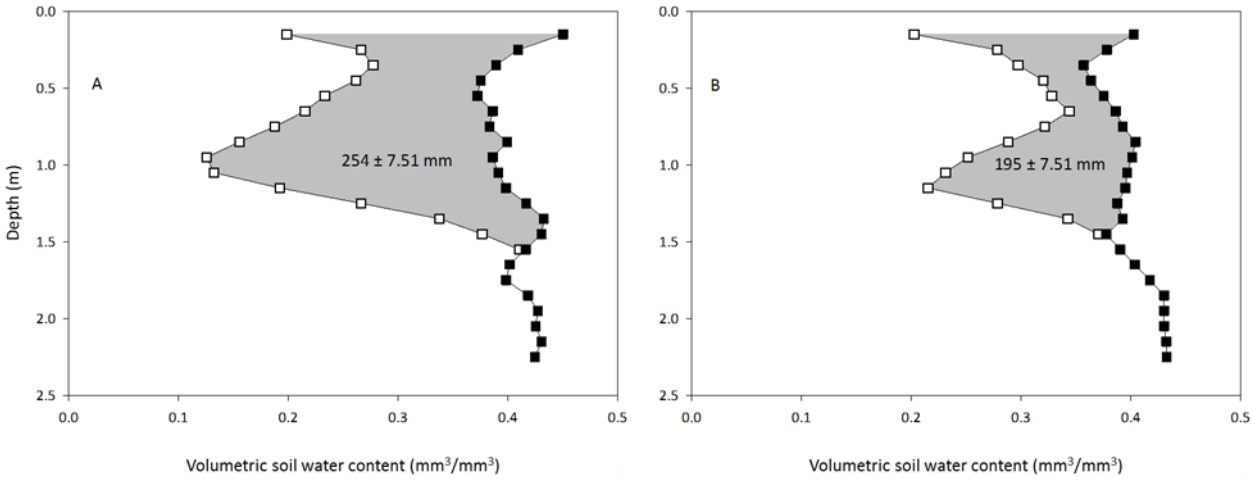


Figure 5-1 Drained upper limits (■) and lower limits (□) of a monoculture of cocksfoot grown at Ladbrooks, Canterbury, New Zealand. Data are for (A) +N pasture (Plot 26, Replicate 1) and (B) −N pasture (Plot 30, Replicate 1). The shaded areas show the plant available water content of the soil within soil layers (0-1.55 ± 0.629 m) for +N pastures and (0-1.45 m) for −N pastures. The text indicates the amount of plant available water content ± SEM (mm).

Experiment 2 at Ashley Dene

From 8/7/2015 to 21/4/2016, the PAWC which contributed to growth was 101 ± 6.85 mm and was not different among species (P=0.171) and N (P=0.119) treatments. The mean estimated maximum depth of water extraction for brome and cocksfoot was 0.855 ± 0.25 m and was deeper (P<0.05) than for perennial ryegrass and tall fescue (0.80 m).

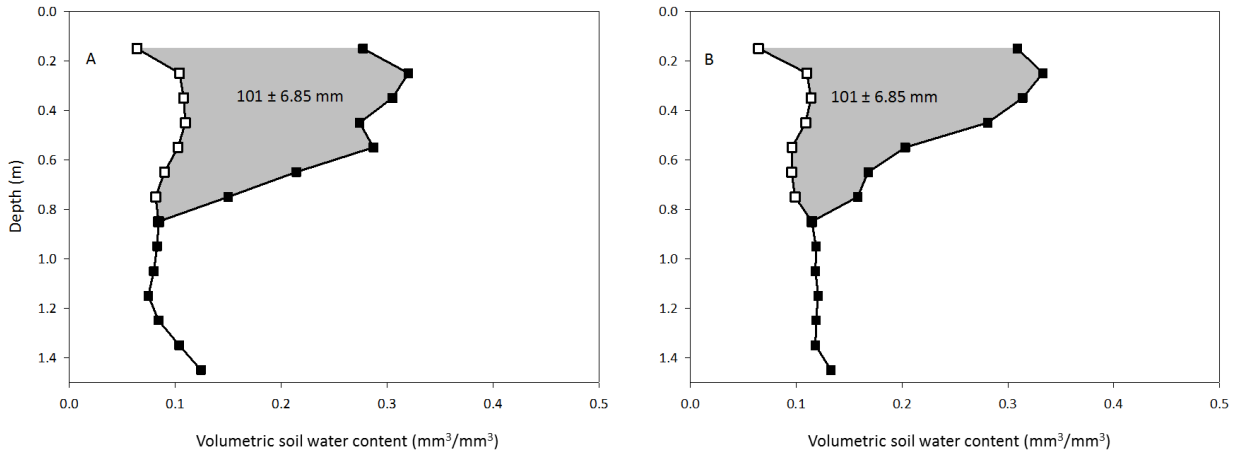


Figure 5-2 Drained upper limits (■) and lower limits (□) of a monoculture of cocksfoot grown at Ashley Dene, Canterbury, New Zealand. Data are for a (A) +N pasture (Plot 26, Replicate 1) and (B) −N pasture (Plot 30, Replicate 1). The shaded areas show the plant available water content of the soil within soil layers (0-0.85 ± 0.25 m). The text indicates the amount of plants available water holding capacity ± SEM (mm).

5.3.2 The actual soil moisture deficit (ASMD)

Experiment 1 at Ladbrooks

Repeated measures analysis for the establishment year (from 15/10/2014 to 20/4/2015) showed no difference in ASMD among species (P=0.219) and N treatments (P=0.526) at different measurement times.

In the first measurement taken on 29/1/2015 in the establishment year, dryland pastures at Ladbrooks showed the minimum ASMD of 64.3 ± 9.5 mm when accumulated thermal time was 1224 °Cd. This increased (P<0.001) to the maximum of 114 ± 4.21 and 115 ± 4.39 on 17/2/2016 (1459 °Cd) and 26/3/2015 (1912 °Cd), respectively (Figure 5-3 A and C).

In the second year (2015/16), the main period of recharge occurred in winter (from 21/4/2015 to 1/10/20105) when 270 mm of rain fell (Figure 5-3). Repeated measures analysis gave overall species and N effects and species*time and N*time interactions (P<0.001) for ASMD for dryland pastures at Ladbrooks. At the start of the growth season (before N application) at 630 °Cd (1/10/2015), the mean ASMD was 46.7 ± 8.27 mm and was not different (P=0.78) between + and −N pastures. Actual soil moisture deficit increased (P<0.001) to a maximum of 226 ± 8.34 mm for +N pastures which was higher (P<0.001) than the 177 ± 5.59 mm for −N pastures on 6/3/2016 (2431 °Cd). The first N application at Ladbrooks was on 18/9/2015. However, N did not affect the ASMD (or DM yield) until 24/12/2015 (1465 °Cd). From 24/12/2015 to 10/4/2016, mean ASMD of +N pastures was ~30-60 mm higher (P<0.001) than the control (−N pastures).

In the second year (from 1/10/2015 to 10/4/2016), there was only a difference among species (P<0.05) at 2842 °Cd on 10/4/2016. On 10/4/2016, the mean ASMD of perennial ryegrass pastures was 184 ± 7.92 mm which was lower than tall fescue (222 mm) but not different from cocksfoot and brome (Figure 5-3).

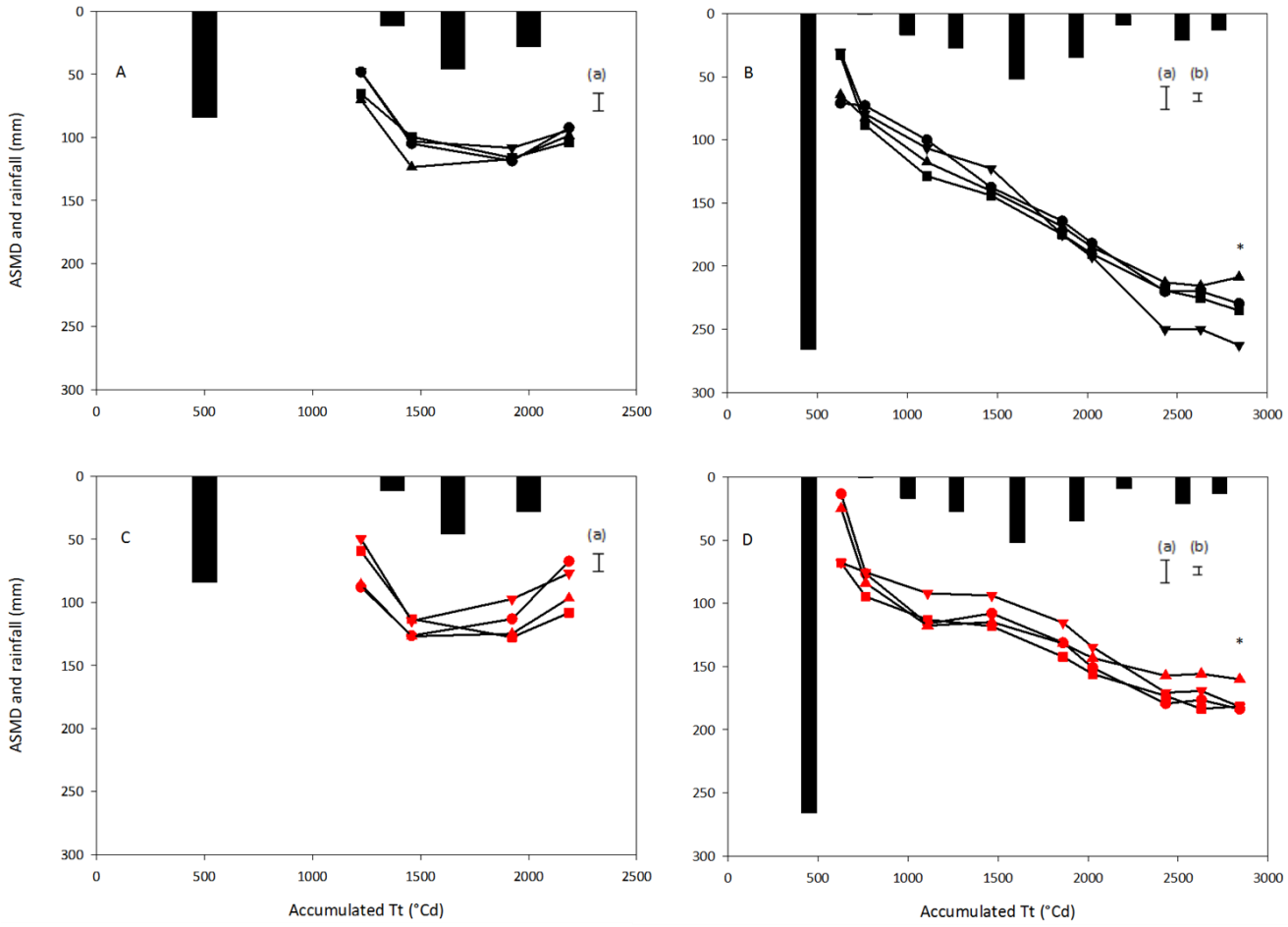


Figure 5-3 Actual soil moisture deficit (ASMD) (mm) from 0.0 – 2.5 m soil depth against accumulated thermal time by +N (A and B) and −N (C and D) brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) in 2014/15 (from 15/10/2014 to 20/4/2015) and 2015/16 (from 1/10/2015 to 10/4/2016) at Ladbrooks, Canterbury, New Zealand. Bar graphs show accumulated rainfall between two measurements. Error bars are maximum SEM for (a) species and (b) N effects. Asterisks shown where differences observed.

Experiment 2 at Ashley Dene

In the second year of the experiment, dryland pastures showed the main period of soil recharge occurred from 8/7/2015 to 29/9/2015. This was after 250 mm of rainfall decreased ASMD of the dryland pasture to the minimum of 32 mm, when accumulated thermal time was 350 °Cd (Figure 5-4) on 29/9/2015. From 29/9/2015 to 11/4/2016 (350-2574 °Cd), there was no difference (P=0.295) in ASMD between + and −N treatments. Mean ASMD for cocksfoot (92.2 ± 3.61 mm) was higher (P<0.05) than perennial ryegrass (80.6 mm) and tall fescue (78.1 mm), but not different from brome (91.2 mm).

Repeated measures analysis showed that, the ASMD was different (P<0.001) at different regrowth cycles. Actual soil moisture deficit increased from 32 mm on 29/9/2015 (350 °Cd) to 107 mm on 15/11/2015 (792 °Cd). On 16/11/2015, the average ASMD was 118 mm which was not different from the ASMD on 15/1/2016 (1450 °Cd). On 5/2/2016, 85 mm of rainfall, decreased the ASMD to 91.1 mm when accumulated thermal time was 1740 °Cd (Figure 5-4).

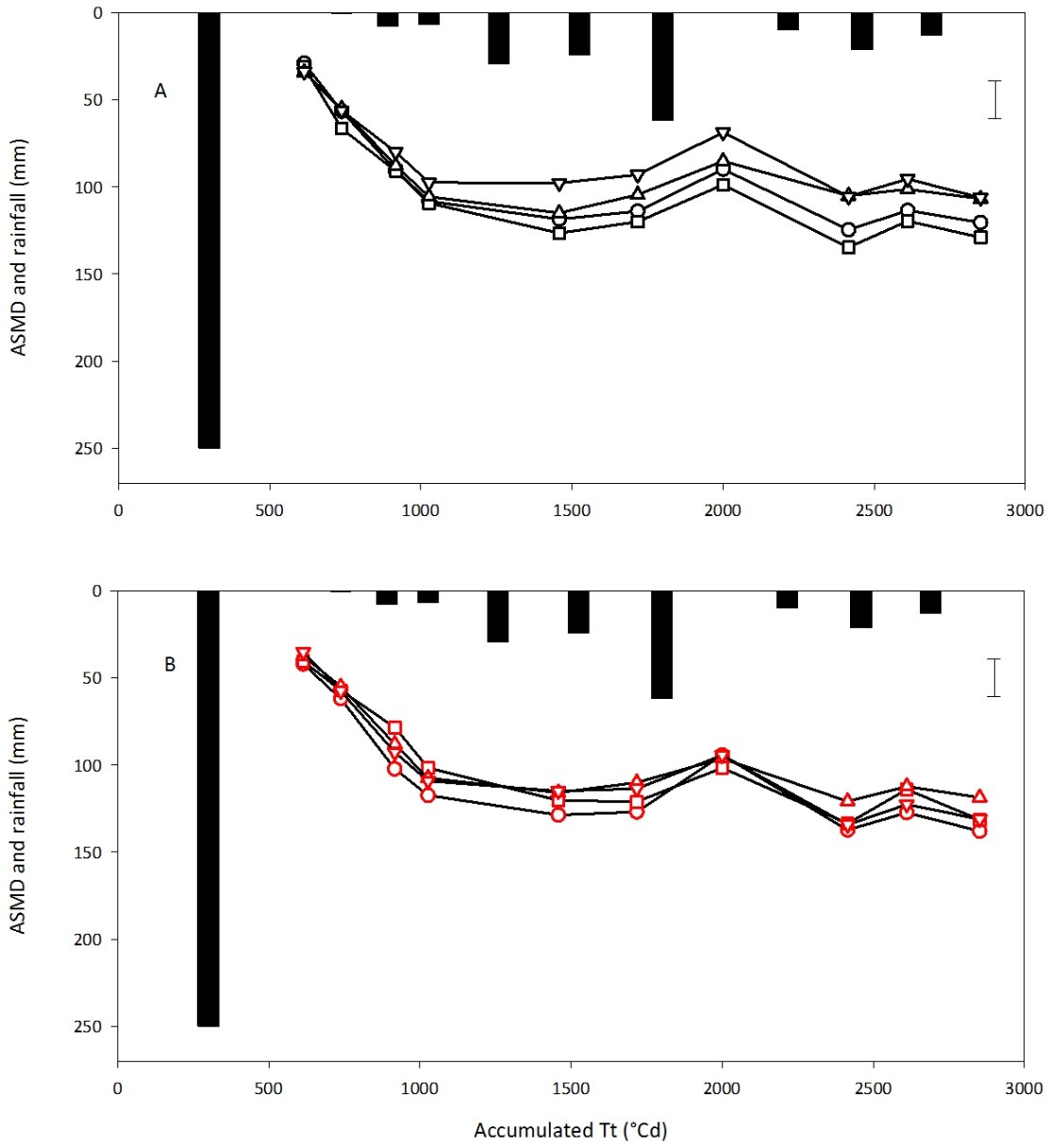


Figure 5-4 Actual soil moisture deficit (ASMD) (mm) from 0.0 - 1.5 m soil depth against accumulated thermal time by +N (A) and −N (B) brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) in 2015/16 (from 29/9/2015 to 11/4/2016) at Ashley Dene, Canterbury, New Zealand. Bar graphs show accumulated rainfall between two measurements. Error bars are the maximum SEM for species.

5.3.3 Water use (WU)

Experiment 1 at Ladbrooks

From 15/10/2014 to 20/4/2015, before N application, mean total water use was 257 ± 3.60 mm and was not different (P=0.108) among species (Table 5-1). Repeated measures analysis showed that, on 31/3/2015, total water used by pastures was higher (P<0.01) than 15/10/2014 and 20/4/2015.

In the second year, from 15/10/2015 to 20/4/2016, mean total water use for +N pastures was 823 ± 9.68 mm and was not different (P=0.437) among species. Total water use by −N pastures (777 mm) was lower (P<0.01) than +N pastures. Repeated measures analysis showed an interaction (P<0.01) between time and N. On 7/2/2016, 14/3/2016 and 20/4/2016, N application did not affect total water use by pastures. ±N pastures also showed the lowest (P<0.001) water use on these dates.

Table 5-1 Total water use (mm) by monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in 2014/15 and 2015/16 at Ladbrooks, Canterbury, New Zealand.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	264	274	269	815	772	793
Cocksfoot	243	246	244	840	782	811
P. ryegrass	256	254	255	799	782	791
Tall fescue	264	256	260	840	772	806
Mean	257	258		823	777	
S	0.108			0.437		
N	0.884			<0.001		
S*N	-			-		
LSD (S)	-			-		
LSD (N)	-			14.7		
LSD (S*N)	-			-		

Note: S and N show the P values for species and nitrogen treatments.

Experiment 2 at Ashley Dene

In the first year, from 17/4/2015 to 7/7/2015 (before applying N), total water use was 191 ± 3.85 mm and not different (P=0.153) among species.

In the second year, from 8/7/2015 to 21/4/2016, mean total water use was 364 ± 3.98 mm and was not different (P=0.190) among species. However, N did not affect (P=0.477) water used by the pastures.

Table 5-2 Total water use (mm) by ±N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in 2015/16 at Ashley Dene, Canterbury, New Zealand.

	2015/16		
	Nitrogen level (N)		
	+N	−N	Mean
Species (S)			
Brome	360	378	369
Cocksfoot	361	352	356
P. ryegrass	362	366	364
Tall fescue	370	363	367
Mean	363	365	
S	0.171		
N	0.532		
S*N	-		
LSD (S)	-		
LSD (N)	-		
LSD (S*N)	-		

Note: S and N show the P values for species and nitrogen treatments.

5.3.4 Water use efficiency (WUE)

Experiment 1 at Ladbrooks

From 15/10/2014 to 20/4/2015, before N application, mean water use efficiency was 13.6 ± 0.64 kg DM ha^{-1} mm^{-1} and was not different (P=0.08) among species (Table 5-3).

In the second year, from 15/10/2015 to 20/4/2016, water use efficiency was not different (P=0.097) among species (Table 5-3). Mean water use efficiency for +N pastures was 19.5 ± 0.30 kg DM ha^{-1} mm^{-1} which was higher (P<0.001) than −N pastures (7.92 kg DM ha^{-1} mm^{-1}).

Table 5-3 Water use efficiency (kg DM ha^{-1} mm^{-1}) of ±N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in 2014/15 and 2015/16 at Ladbrooks, Canterbury, New Zealand.

	2014/15			2015/16		
	Nitrogen level (N)			Nitrogen level (N)		
	+N	−N	Mean	+N	−N	Mean
Species (S)						
Brome	12.0	14.4	13.2	19.9	6.36	13.1
Cocksfoot	12.2	12.3	12.3	19.5	8.14	13.8
P. ryegrass	16.0	11.4	13.7	19.8	9.21	14.5
Tall fescue	15.4	15.0	15.2	18.6	7.99	13.3
Mean	13.9	13.3		19.5	7.92	
S	0.08			0.097		
N	-			<0.001		
S*N	-			-		
LSD (S)	-			-		
LSD (N)	-			1.20		
LSD (S*N)	-			-		

Note: S and N in Table 5-3 above show the P values for species and nitrogen treatments.

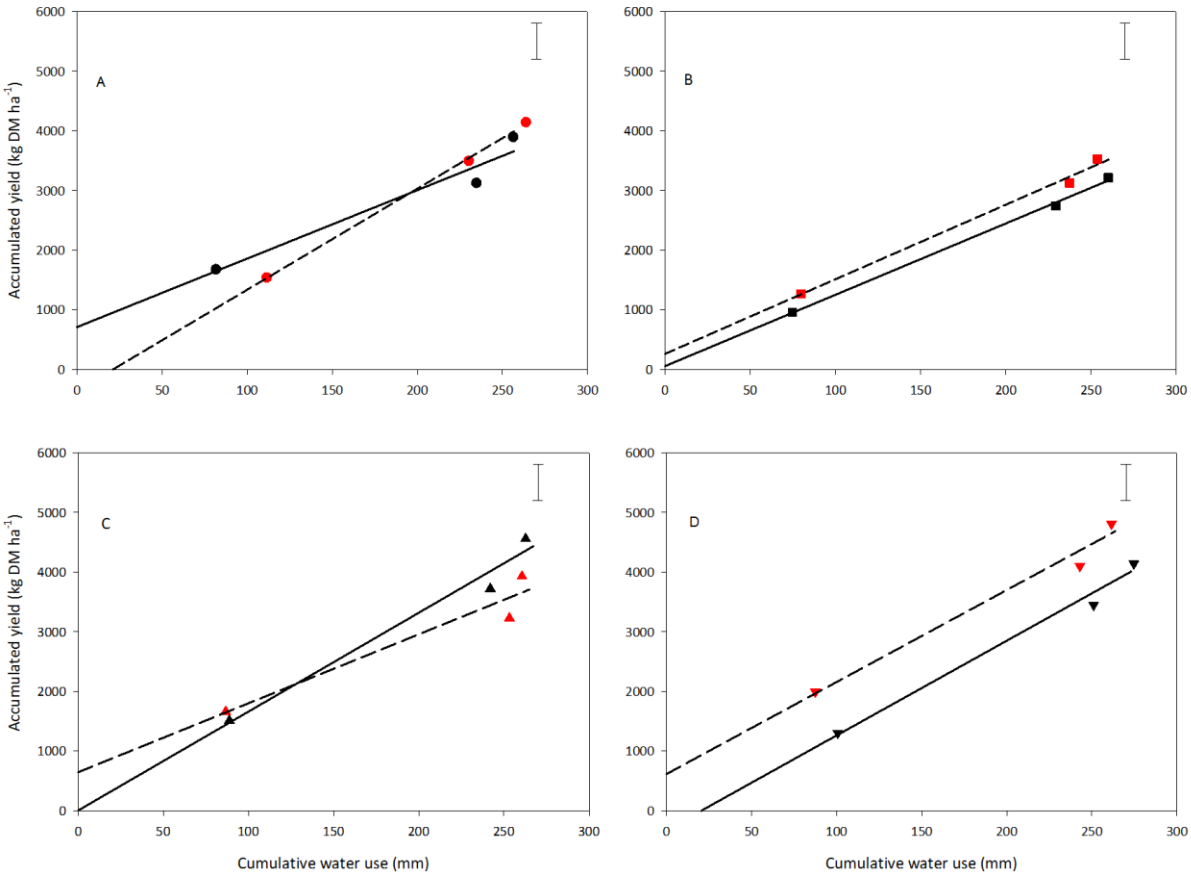


Figure 5-5 Relationship between accumulated yield (kg DM ha^{-1}) and cumulative annual water use (mm) by +N (black) and −N (red) of monocultures of (A) brome (●,●), (B) cocksfoot (■,■), (C) perennial ryegrass (▲,▲) and (D) tall fescue (▼,▼) in 2015/16 at Ladbrooks, Canterbury, New Zealand. Regression equation details for +N (—) and −N (– – –) treatments are reported in Table 5-4. Values are the average accumulated DM measured from 15/10/2014 to 20/4/2015. Error bars are maximum SEM for species.

Table 5-4 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by ±N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in 2014/15 at Ladbrooks, Canterbury, New Zealand.

Species (S)	Equation (Y=)	SE Coef (x)	SE Coef (constant)	R^2
Nitrogen level (N)			+N	
Brome	12.0x+648	3.19	667	0.87
Cocksfoot	12.2x+245	0.84	159	0.99
P. ryegrass	16.0x+342	2.05	413	0.97
Tall fescue	15.4x+27.5	1.44	300	0.98
Nitrogen level (N)			−N	
Brome	14.4x+208	2.09	454	0.96
Cocksfoot	12.3x+817	0.63	122	0.99
P. ryegrass	11.4x+846	2.59	525	0.99
Tall fescue	15.0x+897	1.7	343	0.98

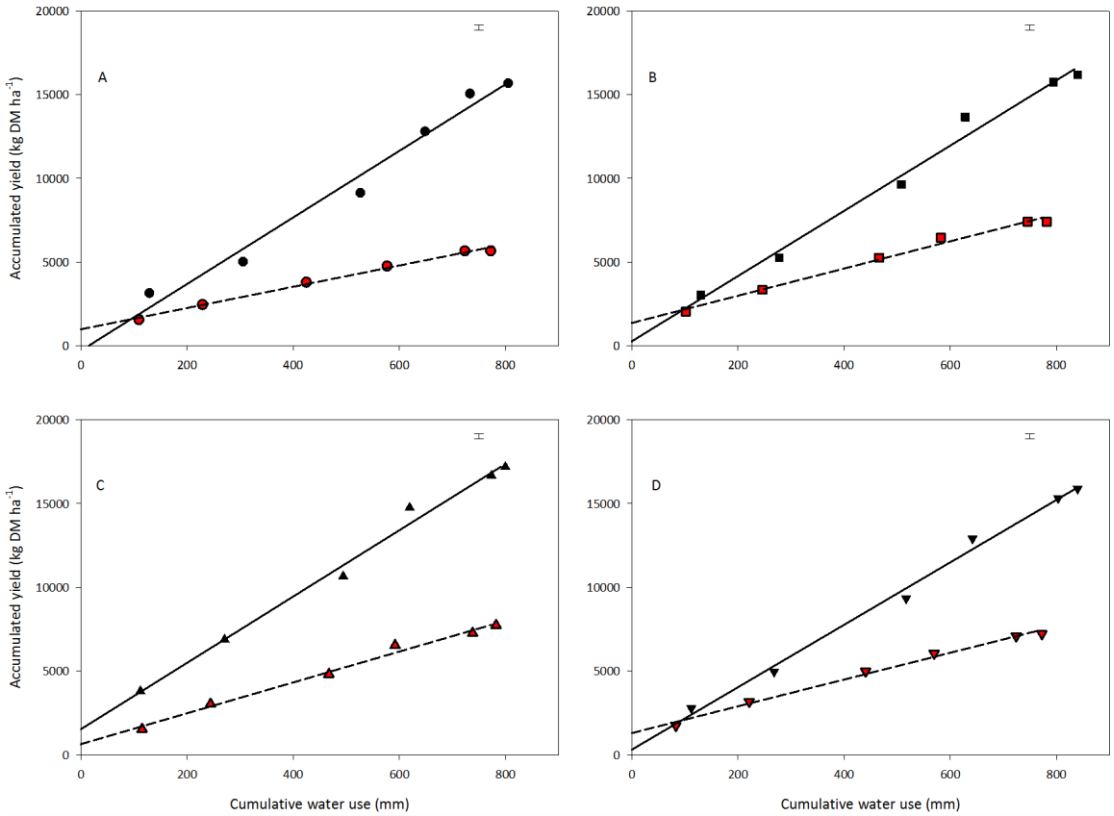


Figure 5-6 Relationship between accumulated yield (kg DM/ha) and cumulative annual water use (mm) by +N (black) and −N (red) of (A) brome (●,●), (B) cocksfoot (■,■), (C) perennial ryegrass (▲,▲) and (D) tall fescue (▼,▼) pastures in 2015/16 at Ladbrooks, Canterbury, New Zealand. Error bars are maximum SEM for N. Regression equation details for +N (—) and −N (---) treatments are reported in Table 5-5. Values are the average accumulated DM measured from 15/10/2015 to 20/4/2016. Error bars are maximum SEM for N.

Table 5-5 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by ±N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in 2015/16 at Ladbrooks, Canterbury, New Zealand.

Species (S)	Equation (Y=)	SE Coef (x)	SE Coef (constant)	R^2
Nitrogen level (N)		+N		
Brome	19.9x-303	1.50	864	0.98
Cocksfoot	19.5x+236	1.13	666	0.99
P. ryegrass	19.8x+1528	0.967	550	0.99
Tall fescue	18.6x+305	0.780	462	0.99
N level (N)		−N		
Brome	6.36x+1092	0.199	106	0.99
Cocksfoot	8.14x+1349	0.42	227	0.99
P. ryegrass	9.21x+638	0.457	250	0.99
Tall fescue	7.99x+1298	0.356	189	0.99

Experiment 2 at Ashley Dene

From 8/7/2015 to 21/4/2016, there was an interaction (P<0.01) between species and N. Nitrogen only increased water use efficiency of cocksfoot and brome. Water use efficiency of ±N cocksfoot was higher (P<0.01) than other species. The average water use efficiency of ±N perennial ryegrass (13.6 ± 0.89 kg DM ha^{-1} mm^{-1}) was higher than tall fescue but not different from brome (Table 5-6).

Table 5-6 Water use efficiency (kg DM ha^{-1} mm^{-1}) of ±N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in 2015/16 at Ashley Dene, Canterbury, New Zealand.

	2015/15		
	Nitrogen level (N)		
	+N	−N	Mean
Species (S)			
Brome	12.7	9.81	11.3 b
Cocksfoot	20.5	12.8	16.6 a
P. ryegrass	14.0	13.2	13.6 b
Tall fescue	9.0	11.1	10.1 c
Mean	14.1	11.7	
S	0.003		
N	0.009		
S*N	0.004		
LSD (S)	2.83		
LSD (N)	1.64		
LSD (S*N)	3.44		

Note: S, N and S*N show the P values for species, nitrogen and species*N interactions. Means within a column with different letters are significantly different (LSD at the α≤0.05 level).

Figure 5-7 shows the relationship between accumulated yield (kg DM ha^{-1}) and cumulative annual water use (mm). There was an average of 118 ± 9.7 mm for the interception of the linear regressions fitted to data on the x-axis. Accumulated highest SMC to the maximum depth of extraction (0.85 m) at Ashley Dene was between 100 to 120 mm which was measured after 216 mm of rainfall (Section 5.2.4). Therefore, the estimated amount of drained water from the soil profile at Ashley Dene is between 100 to 120 mm.

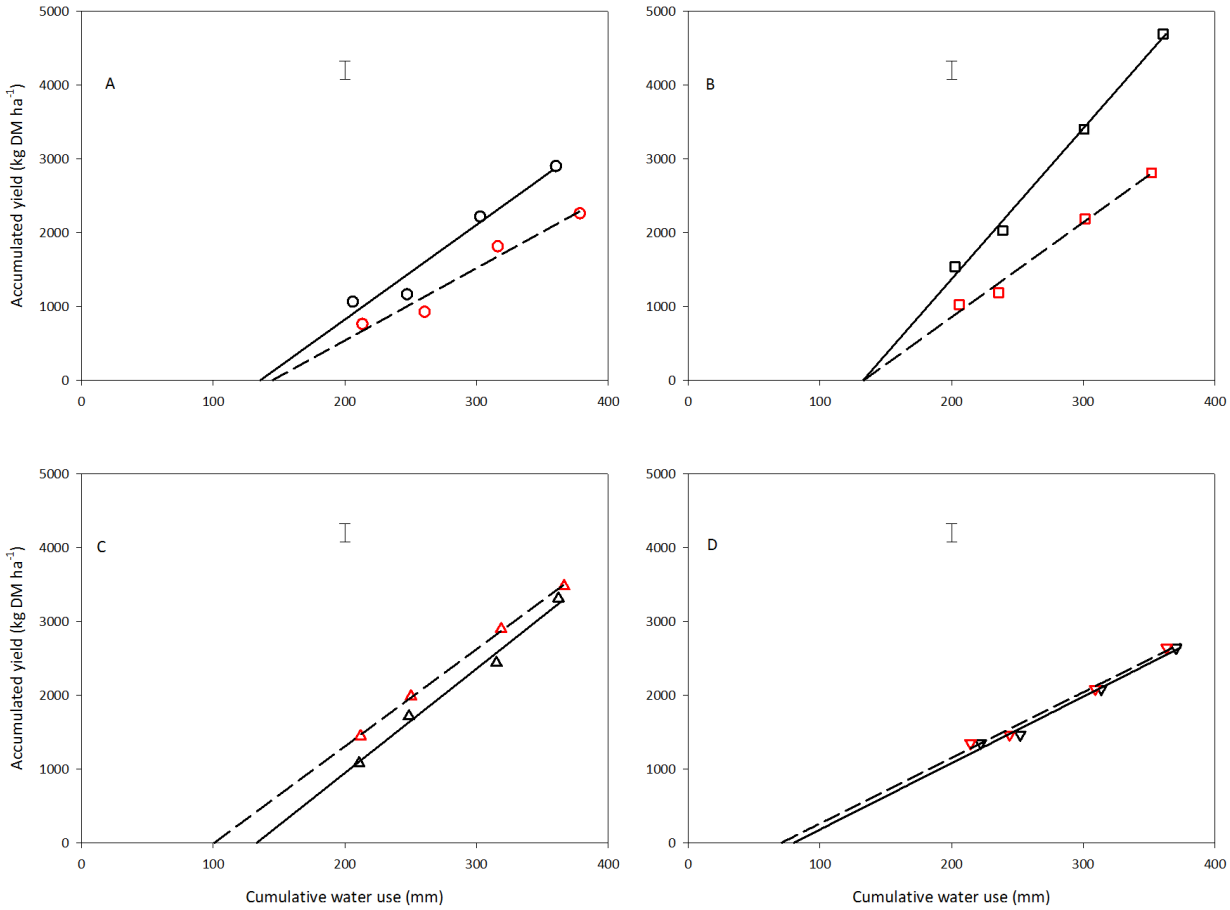


Figure 5-7 Relationship between accumulated yield (kg DM ha^{-1}) and cumulative annual water use (mm) by +N (black) and −N (red) of (A) brome (○,○) , (B) cocksfoot (□,□), (C) perennial ryegrass (△,△) and (D) tall fescue (▽,▽) in 2015/16 at Ashley Dene, Canterbury, New Zealand. Error bars are maximum SEM for species. Regression equation details for +N (—) and −N (- - - -) treatments are reported in Table 5-7. Values are the average accumulated DM measured from 8/7/2015 to 21/4/2016. Error bars are maximum SEM for species.

Table 5-7 Regression equations for accumulated yield (kg DM ha^{-1}) against accumulated water use (mm) by ±N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue in 2015/16 at Ashley Dene, Canterbury, New Zealand.

Species (S)	Equation (Y=)	SE Coef (x)	SE Coef (constant)	R^2
N level (N)			+N	
Brome	12.8x-1745	1.96	558	0.96
Cocksfoot	20.4x-2713	1.07	302	0.99
P. ryegrass	14.1x-1884	1.056	306	0.99
Tall fescue	9.00x-723	0.708	209	0.99
N level (N)			−N	
Brome	9.81x-1425	1.52	454	0.95
Cocksfoot	12.8x-1714	1.01	282	0.99
P. ryegrass	13.2x-1330	0.246	72	0.99
Tall fescue	8.90X-632	0.719	208	0.99

5.3.5 Critical soil moisture deficit

To determine the critical soil moisture deficit, the occurrence and timing of moisture stress was inspected using the relationship between water use/EPT and ASMD. The method of using ASMD to quantify moisture stress and define a critical limiting deficit (DL), the point where yield reductions occurred (Penman, 1971) did not give a clear value for the pastures both at Ladbrooks and Ashley Dene. From 1/10/2015 to 20/4/2016 at Ladbrooks, on only one occasion (on 1/10/2015), water use/EPT reached 1.0 (Figure 5-8). This suggests the soil could only fully meet crop demand after winter rainfall. At Ladbrooks, since pasture production was similar among species but different between + and −N treatments, in Figure 5-8 only + and −N treatments are separated. Horizontal lines are the average water use/PET, for the measurements taken from 1/10/2015 to 6/3/2016, in Phase 1 (Section 4.11.11), before the onset of more severe moisture stress. This was 0.75 for +N pastures and 0.69 for −N pastures. Beyond this level water use/EPT was reduced by 0.005 per mm of ASMD for +N pastures and 0.0095 for −N pastures (Figure 5-8).

Water use/PET decreased to 0.45±0.013 when ASMD increased to 295 mm. Water use/PET decreased to 0.36±0.045 for −N pastures when ASMD was 161 ± 9 mm.

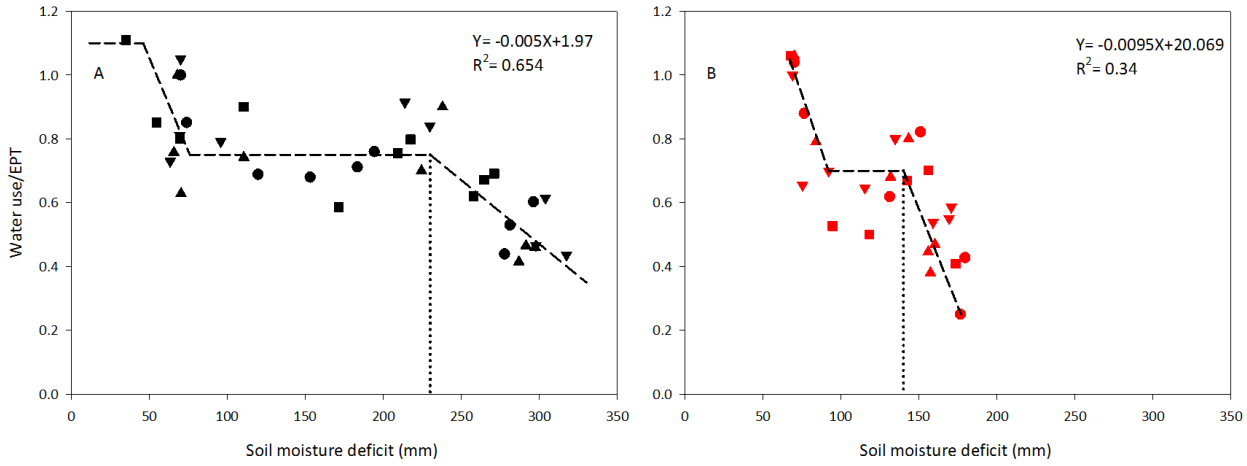


Figure 5-8 The mean soil moisture deficit in relation to the moisture stress (ET/EPT) for individual regrowth cycles of +N (black) and −N (red) of (A) brome (●,●), (B) cocksfoot (■,■), (C) perennial ryegrass (▲,▲) and (D) tall fescue (▼,▼) pastures in 2015/16 at Ladbrooks, Canterbury, New Zealand. Dashed lines have set on the average water use/EPT for + (A) and −N (B) pastures. Dotted lines indicate the point where yield reductions occurred.

From 12/10/2015 to 11/4/2016 at Ashley Dene, water use/EPT did not reach 1.0, with an average maximum of 60% of crop water demand provided by the soil (Figure 5-9). At Ashley Dene, water use/EPT was at the maximum of 0.6 for brome and cocksfoot, when ASDM was between 50-110 ± 9.5 mm. Water use/EPT was at the maximum of 0.6 for tall fescue and perennial ryegrass when ASDM was between 50-80 ± 12 mm.

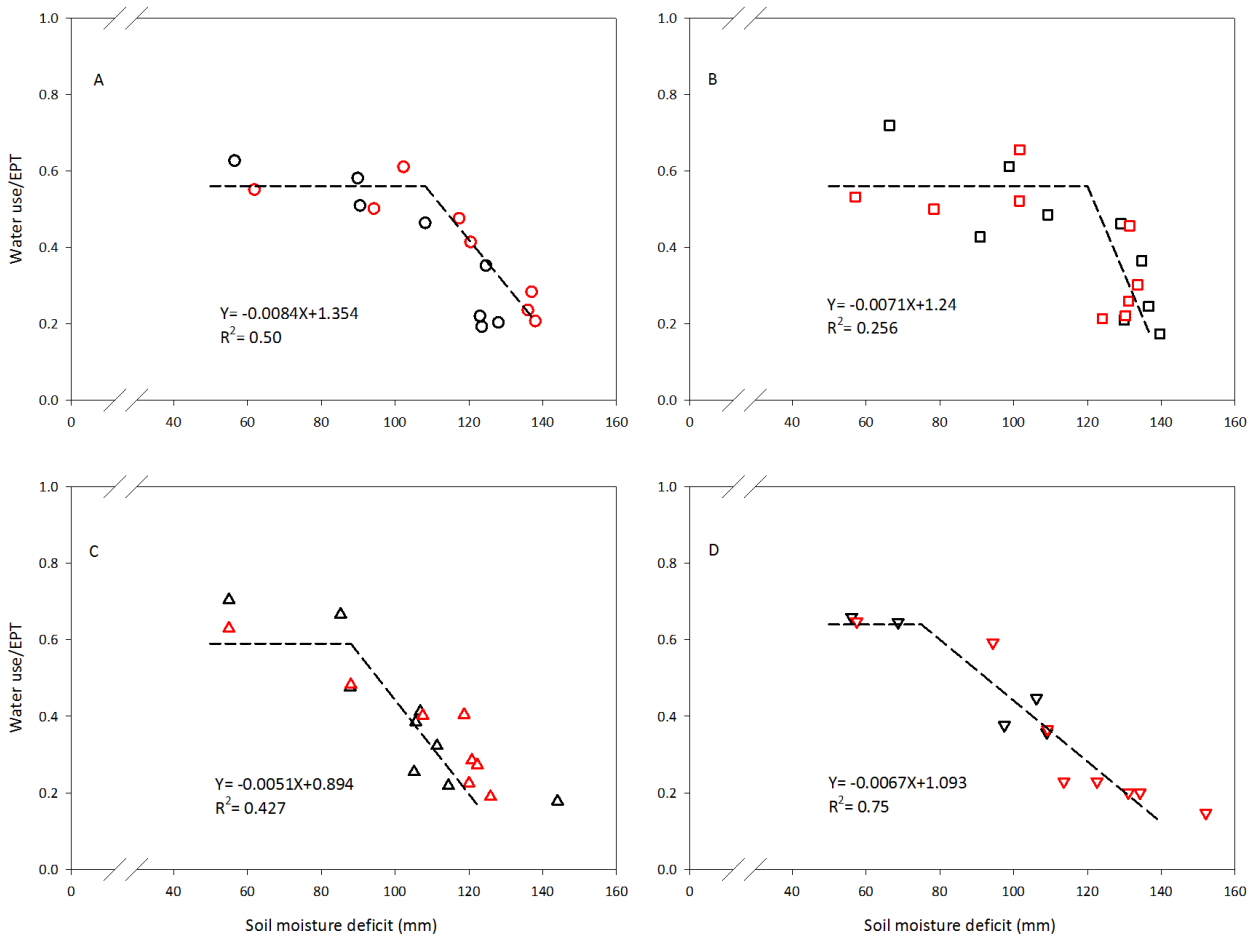


Figure 5-9 The mean actual soil moisture deficit in relation to the moisture stress (ET/EPT) for individual regrowth cycles by +N (black) and −N (red) of (A) brome (○,○) , (B) cocksfoot (□,□), (C) perennial ryegrass (△,△) and (D) tall fescue (▽,▽) in 2015/16 at Ashley Dene, Canterbury, New Zealand. Dashed lines have set on the average water use/EPT.

ET/EPT did not reach 1.0 at Ashley Dene and only in few cases reached to 1.0 at Ladbrooks. Therefore, the theory of critical soil moisture deficit could not be used in this study for the dryland monoculture grasses sown in Wakanui silt loam and Lismore stony silt loam soil types in 2015/16.

5.4 Discussion

Chapter 4 showed, DM production of + N pastures was more than double the control at Ladbrooks. Also, when grown in a Lismore stony silt loam soil pasture at Ashley Dene, the DM production of +N cocksfoot was almost double the other species used in this study. In this chapter the differences in annual DM production between two sites reported in Chapter 4 were investigated in relation to their moisture and N levels. To do this, required first for PAWC to be quantified at each site and how N application affected it to be defined (Objective 5, Section 1.4). Then changes in ASDM over the duration of the study in response to species and N were described (Objective 6).

Accumulated Tt was applied to account for the effect of seasonal temperature on pasture production (Section 4.11.11). Previous studies (Hutchinson *et al.,* 2000) also defined long-term DM (1950-1961) production (Radcliffe, 1974) of a mixed species pasture. The relationships presented in the literature usually exclude periods when either moisture, or N were limited. When N and moisture are non-limited, there is a linear relationship between accumulated thermal time and accumulated DM (Mills, 2006). However, under dryland conditions, once N and moisture restrict pasture production, the linearity fails (Section 4.11.11). The superior production and recovery after moisture stress by +N pastures at Ladbrooks and +N cocksfoot at Ashley Dene compared with the other species may reflect differences among species in their ability to access and extract soil moisture and/or water use efficiency. Therefore, Objective 7 (Section 1.4) was to compare water use and water use efficiency of these dryland pastures under different levels of moisture and N.

5.4.1 Plant available water content and actual soil moisture deficit

PAWC is the result of both soil and root features (Jamieson and Ewert, 1999). Soil texture affects the soil pore size distribution which regulates the amount of moisture that the soil is able to store. Root characteristics of the plants also affect PAWC (Section 2.1). Objective 5 was to quantify PAWC of the soil types at different sites. The effect of N on PAWC was also investigated.

In this study, the major difference between the two sites, was the different soil types of Wakanui silt loam at Ladbrooks and Lismore stony silt loam soil at Ashley Dene. These are derived from similar parent material and soil texture and pore size are comparable. The higher stone content and shallow top soil of the Lismore stony silt loam soil at Ashley Dene, resulted in an average DUL volumetric water content of 0.187 mm^3/mm^3 to a depth of 1.45 m compared with 0.401 mm^3/mm^3 to the same depth at Ladbrooks (Figure 5-1 and 5-2).

In established pastures in 2015/16 at Ladbrooks, the maximum depth of root water extraction from the deep Wakanui silt loam soil was almost double that at Ashley Dene from the shallow stony soil (Figure 5-1 and 5-2). Therefore, soil depth and texture affected moisture availability for these pastures with 45% lower PAWC at Ashley Dene compared with Ladbrooks. At Ashley Dene, PAWC was not different among species or between + and −N pastures (Figure 5-2). Mills (2007) reported that, while production of dryland monoculture 'Wana' cocksfoot was almost doubled in +N (15.7 t DM/ha yr^{-1}) compared with −N treatment (5-7.5 t DM/ha yr^{-1}), PAWC was not different between + and −N treatments. The results for the maximum depth of extraction of brome and cocksfoot at Ashley Dene with a Lismore stony silt loam soil were similar to the established 'Wana' cocksfoot on a Templeton silt loam soil with a similar soil depth (Mills, 2007). However, due to the different soil texture of Templeton silt loam soil, PAWC was 55 mm more than pastures at Ashley Dene and ~100 mm less than +N pastures at Ladbrooks.

In this research, the average estimated depth of moisture extraction for brome and cocksfoot was 8% more than perennial ryegrass and tall fescue at Ashley Dene. Nonetheless, this did not make any difference in PAWC. Evans (1978) showed that, on a sandy loam soil with a 4.0 m depth above parent material on a river terrace in Palmerston North, New Zealand, cocksfoot produced twice as much herbage as ryegrass and white clover. However, it did not extract moisture from a greater depth in the soil. Considering the results of previous studies and the current study in terms of the maximum depth of root water extraction of two pastures with very different soil depth, there was no advantage among these grass species in terms of using soil moisture stored in the deeper soil layers for each soil type.

Figure 4-19 (Chapter 4, Section 4.11.11) showed that, there was a two phase linear relationship between accumulated thermal time (°Cd) versus accumulated DM (kg DM ha^{-1}) fitted to + and −N data at Ladbrooks. The linearity in the relationship between accumulated thermal time versus DM stopped in summer, once accumulated thermal time by + and −N pastures was 2298 ± 64.4 °Cd and 1877± 49.9 °Cd, respectively (Phase 2). Figure 5-3 showed that, for + N pastures this was when the ASDM was almost constant at 229 ±2.53 mm, once accumulated thermal time was between 2431 and 2842 °Cd and accumulated rainfall was <50 mm (Figure 5-3). Plotting water use/EPT against ASDM (Figure 5-8), also showed that, once ASDM>230 mm for +N pastures, water use/ EPT decreased by 0.005 per mm of soil moisture deficit. This occurred at ~2300 to 2431 °Cd for +N pastures. Plotting water use/EPT against ASDM also showed that, once ASDM>140 mm for −N pastures, water use/ EPT decreased by 0.0095 per mm of soil moisture deficit. For −N pastures this was when the average soil moisture deficit was ≤146±4.6 mm (Figure 5-3) when accumulated thermal time was between ~1860 to 1877 °Cd. The results suggest that, for +N pastures at Ladbrooks, in Phase 1 (Figure 4-19), on average 75% of crop water demand was provided by the soil, once the ASDM ≤230 mm for +N pastures. On average 69% of

crop water demand was provided by the soil For −N pastures in Phase 1, when the average ASDM was ≤146±4.6 mm.

Production and the ability of pasture for recovery after moisture stress are important factors that affect dryland pastures. Under dryland conditions, maximum utilization of rainfall events occurs when the available soil moisture is above the point where recovery rate declines. If rainfall could not meet this requirement, then use of more drought tolerable species may result in improved WUE and DM production throughout summer.

Once pasture was extremely moisture stressed at Ashley Dene (between 15/11/2016 to 11/4/2016), 85 mm of rainfall in the middle of January decreased the average ASDM by ~40 mm in both + and −N pastures (Figure 5-4). Under these conditions, +N cocksfoot pasture at Ashley Dene recovered and produced double the DM compared with the other ±N pastures in January 2016 (Figure 4-7). The results of this chapter showed that an ASDM of 50-80 mm was the point at which water use/EPT for perennial ryegrass and tall fescue declined. However, under the same conditions, recovery of brome and cocksfoot pastures was faster than the other species and improved WUE and DM production. Turner *et al*. (2012) found that, in cocksfoot plants, the unique form of water-soluble carbohydrate reserve depletion and replenishment in the recovery phase maintained more stable DM production which enabled cocksfoot to be more drought tolerant than perennial ryegrass and tall fescue. A glasshouse experiment, comparing perennial ryegrass, cocksfoot and tall fescue showed that, tall fescue was able to recover on re-watering more rapidly than the other two other species. Under a moderate drought stress (33% of the optimum moisture), tall fescue showed a modest drought response and was able to produce the highest DM and had a robust capability of recovery on re-watering. However, care is needed in comparing results from the experiments run under controlled environments to field experiments, mainly because of the restricted rooting depth in pots compared with the field (Sheehy *et al*., 1975; Norris and Thomas, 1982), and potential differences in evapotranspiration which is a result of differences in light intensity. In the current study, tall fescue did not show any advantage in terms of a deeper rooting system or extracting more moisture at either site. The only difference occurred on 10/4/2016, when ASMD of +N tall fescue was higher than other species at Ladbrooks. However, DM production of tall fescue was similar and therefore WUE was less than the other +N species. Under more severe moisture stress at Ashley Dene, tall fescue showed no more stress tolerance than perennial ryegrass and brome. Tall fescue response to 85 mm of rainfall was similar to brome and perennial ryegrass. This lack of response may be either due to the poor pasture establishment and low tiller density of tall fescue at Ashley Dene (field observation) or plants inability to recovery after a period of moisture stress. West *et al.* (1993) found that, tall fescue recovery is related to the level of endophyte infection and tiller density. Endophyte-free tall fescue showed a

poor recovery after moisture stress. Since pastures were endophyte-free in this research, further investigation is required to understand the effect of endophyte on tall fescue production and persistence under moisture stress.

Reduced carbohydrate storage by perennial ryegrass and tall fescue under drought stress has been previously reported (Norris and Thomas, 1982; Karsten and MacAdam, 2001). This ascribed to the use of WSC for cell maintenance and plant growth. Depleted stubble WSC reserves contribute to the poor pasture persistence in summer dry environments (Waller and Sale, 2001). This might be one of the reasons that caused failure in production of tall fescue and perennial ryegrass at Ashley Dene.

Objective 7 (Section 1.4) of this study was to calculate water use and water use efficiency of dryland pastures under different levels of moisture and N.

5.4.2 Water use and water use efficiency

Before application of N in 2014/15, water use (Table 5-1) and WUE (Table 5-3) were not different among species or between ±N plots. In 2015/16, total water use by +N pastures at Ladbrooks was only 2-10% higher than −N pastures. Differences in the WUE were mainly caused by N. N application caused a similar response for each species and resulted in a 55-65% increase in WUE of the pastures at Ladbrooks. Nitrogen affected WUE indirectly because photosynthetic capacity for +N pastures was increased (Peri, 2002) relative to −N pastures.

Comparing the different grasses between the two sites shows that, in 2015/16, WUE of +N tall fescue, brome and perennial ryegrass at Ashley Dene was 35-55% lower than that at Ladbrooks (Tables 3-5 and 3-6). At the same time, WUE of +N cocksfoot was similar between the two sites. Total WU was not different for all pasture species at Ashley Dene. However, the efficiency with which the water was used will be intrinsically linked to the increase in radiation interception caused by N fertiliser (Chapter 6). This shows the ability of cocksfoot to use N fertiliser more efficiently than other grasses at Ashley Dene, when nutrient uptake by the other species was restricted by severe moisture stress. At both sites, advantages in the annual WUE of cocksfoot were predominantly caused by N.

The amount of water used by brome pastures was not different to other pasture grasses both at Ashley Dene and Ladbrooks. The results showed a positive response in WUE for brome and cocksfoot but not for perennial ryegrass and tall fescue pastures at Ashley Dene. However, WUE of +N brome was not different to ±N perennial ryegrass. In a cool temperate environment, perennial grasses that can tolerate dry summer and/or low winter temperatures may be useful. Under this condition, cocksfoot is not likely to offer an advantage over perennial ryegrass and tall fescue because of limited DM production. Moot (2011), showed the ability of ‘Bareno’ brome treated with N fertiliser to produce as

much DM as cocksfoot, three, four and five years after sowing at Lees valley. Brome, cocksfoot and hybrid ryegrass ‘Revolution’ had the highest summer/autumn production. However, there is no literature about root water extraction and WUE of brome.

5.5 Conclusions

In the current chapter, the results deal with Objectives 5 and 6 (Section 1.4). Based on the results, the following conclusions can be made:

- PAWC was not different among species at each site. At Ladbrooks, N caused a 25% increase in PAWC of dryland brome, cocksfoot, perennial ryegrass and tall fescue.

- PAWC was 45-60% lower at Ashley Dene with a Lismore stony soil compared with Ladbrooks with a deep Wakanui silt loam soil.

- At Ashley Dene, N application did not cause any difference in PAWC among species. +N cocksfoot production was two times higher than the other species while the amount of extracted water was similar to the other sown grasses.

- Nitrogen application caused a ≤10% increase in water use and a 55-65% increase in WUE of the pastures at Ladbrooks.

- At Ashley Dene, WUE of ±N perennial ryegrass and tall fescue was not different. WUE of +N cocksfoot was two times higher than the other grasses. Therefore, higher production of +N cocksfoot compared with the other species at Ashley Dene was due to a double WUE but not water use.

The following chapter describes the cause of yield differences on dryland pastures by explaining differences in light interception and light use efficiency caused by N and moisture availability.

Chapter 6 Light interception and radiation use efficiency

Chapter 4 showed the differences in herbage DM yield of brome, cocksfoot, perennial ryegrass and tall fescue monocultures under two different soil types and N levels. Chapter 5 showed, the difference in DM production of the pastures between two sites was due to the difference in PAWC of the different soil types at Ladbrooks and Ashley Dene. Yield differences can be explained as the result of differences in growth, which are the product of available total solar radiation (Ro), multiplied by intercepted light by plants green leaves (R/Ro) and its efficiency of conversion to biomass (radiation use efficiency; RUE) as indicated previously in Equation 2-1 (Monteith, 1977; Gallagher and Biscoe, 1978).

The effect of environmental variables on DM production of a specific crop can be broken into four levels (De Wit, 1986). The first level is the maximum production for that crop in a region, which is determined by local available total solar radiation (Ro) and temperature effects on radiation interception and radiation use efficiency (Equation 2-1). However, both R/RO and RUE can be affected by the second level which is water limitation. Under non-limiting moisture conditions, there is a linear relationship between DM yield and intercepted PAR, the slope of which is the RUE (Monteith, 1972, 1977; Sinclair and Muchow, 1999b). Levels three and four are defined by mineral availability/toxicity and these are a consequence of the soil conditions in which a crop is grown (Fageria *et al*., 1997). Under field conditions, the combination of all four levels of limitation contribute to site and season to determine the yield of any crop or pasture.

Radiation interception is driven by the ability of a crop or pasture to expand its canopy. This is quantified as leaf area index (LAI) which is a measure of green leaf area per unit of ground area. To quantify yield formation components, in this chapter canopy expansion (quantified by LAI) and light interception were calculated (Objective 8, Section 1.4). Once light interception has been quantified, any further yield differences can be related to RUE. Therefore Objective 9 was to define whether the yield differences reported in Chapter 4 resulted from differences in radiation interception and/or RUE.

6.1 Materials and Methods

Details of experimental design and treatments (Section 4.7.1), soil test results (Section 4.7.4), weed management (Section 4.7.5), mowing and grazing (Section 4.7.6) and N fertiliser application (Section 4.7.7) were presented in Chapter 4. Materials and methods to quantify radiation interception of the ±N grass species at both sites (Ladbrooks and Ashley Dene) are detailed below.

6.1.1 Fractional radiation interception at harvest

R/Ro was measured directly by non-destructive measurements using a Sunscan plant canopy analyser system (Delta-T Devices Ltd., Burwell, Cambridge, England) using Equation 6-1:

Equation 6-1 $PAR_i = R/Ro$

Where R is intercepted PAR and Ro is incident PAR in MJ PAR/m^2.

To measure intercepted radiation, below canopy measurements were made at ground level. For each plot, eight below canopy readings were taken by inserting a metal channel (1.0x0.04x0.03m) into a representative area of each plot close to the neutron access tubes and perpendicular to the drill rows at both sites. Incident light was measured by the reference sensor of the sunscan, which measures the direct and diffuse light above the canopy simultaneously. Transmitted photosynthetically active radiation (PAR) is the mean calculated from 64 photodiodes evenly spread out along a 1 m long below canopy sensor. This reduces the need for multiple measurements. Measurements were taken during stable light conditions within two hours either side of solar noon. To monitor the changes in the incident light during the measurement time and also to compare the metal channel numbers with the numbers taken from the reference sensor of the sunscan, four calibrations were made before and after taking measurements of each block. To do that, the metal channel was held approximately 100 mm above the ground level horizontally and four measurements of incident light were taken. The calibration data were then used to account for the changes in incident light by using the "Forecast" function in Excel 2015.

The analyser settings for the leaf absorption parameter was set at 0.85. This is because, most leaves have an absorption value between 0.8 and 0.9. So, an average value of these will usually be appropriate (Webb *et al*., 2008).

The ellipsoidal leaf angle distribution parameter (ELADP) was set at 1.0 which indicates a canopy tending towards randomness. This was assumed because, determining canopy structure/ ELADP at each of the measurement dates was impractical due to the time involved to process samples.

Direct measurement of the canopy parameters in short swards is difficult (Welles and Cohen, 1996; Nouvellon *et al*., 2000; Jonckheere *et al*., 2004) and often inaccurate. Thus, measurements were taken on 14 occasions at the end of each rotation both at Ladbrooks and Ashley Dene throughout the second year (from September 2015 to July 2016).

6.1.2 Estimation of R/RO by the residual biomass

Light interception measurement and estimation of leaf area index (LAI) are usually measured in annual crops where the growth season is shorter and there is no residual biomass because crops are ungrazed. For pastures, it was necessary to also estimate the light intercepted by the pasture residuals to quantify the total amount of intercepted light by the pastures during each regrowth cycle. The logistical problem is compounded by the ongoing defoliation which leaves a residual that is difficult to measure directly. Therefore, an indirect method was developed. To do this a separate calibration was undertaken between the fractional radiation interception (R/RO) by the plants (sunscan measurements) and canopy height from rising plate measurements. Fractional R/RO by the pastures residual was then extrapolated back to the start of the rotation to estimate light interception by residual biomass. An exponential curve were used for calibrations related to canopy height and fractional radiation interception (Figure 6-1).

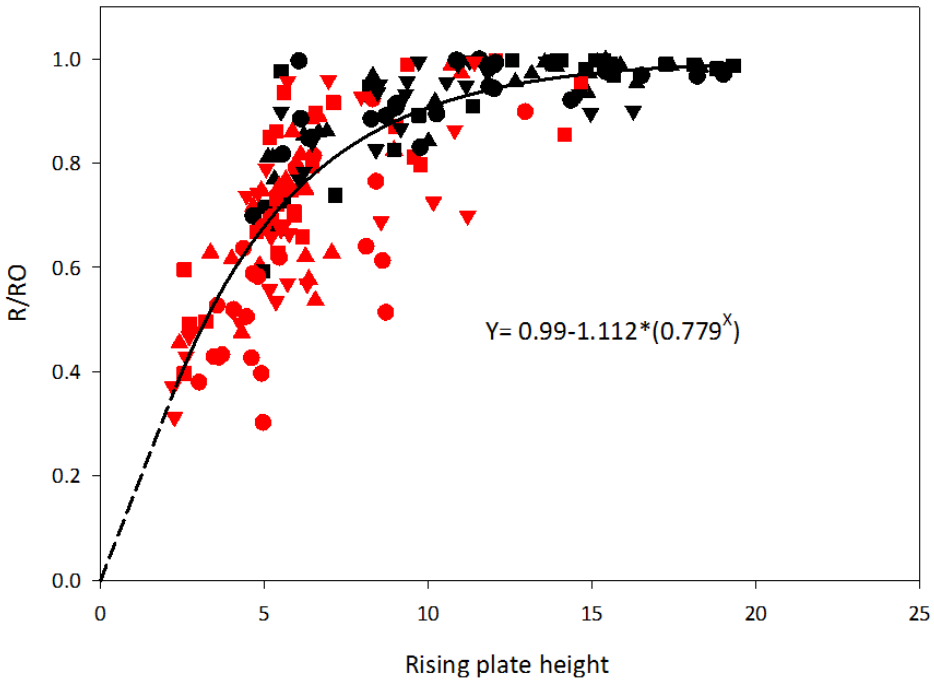


Figure 6-1 Fractional intercepted PAR (R/Ro) against rising plate height in +N (black) and −N (red) monocultures of brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) at Ladbrooks, Canterbury, New Zealand.

6.1.3 Main limitations of using Sunscan

In this study, there were some limitations and constrains in using the Sunscan plant canopy analyser (Delta-T Devices Ltd., Burwell, Cambridge, England). Based on field observations, at Ashley Dene, where pastures were grazed at the end of each regrowth cycle (Appendix E) pastures were not grazed

evenly by animals. This may cause an inaccuracy both in estimation of intercepted light by residual biomass (estimated based on pasture plate height, Section 6.1.2) and also overestimating light intercepted by dead material in not-fully grazed or over grazed pastures. As an example, at Ashley Dene, animals were less interested in grazing −N plots. Thus, the quantity of light intercepted by −N pastures at Ashley Dene might be overestimated.

Stony soils of Ashley Dene also affected light interception measurements. Due to the presence of stones in top of the soil at Ashley Dene, in some cases, it was hard to push the sunscan rode below the canopy and maintain it at ground level.

The lack of differentiation between green and dead material by Sunscan plant canopy analyser might also be more important at Ashley Dene, where the proportion of dead material to total yield for perennial ryegrass was higher (Section 4.11.6) than at Ladbrooks.

6.1.4 Estimation of intercepted PAR in each regrowth cycle

Incident solar radiation (MJ/m^2) was measured hourly using a LiCor pyranometer – CR1000 datalogger installed at the centre of the experimental field (block two) at each site. To calculate daily PAR (MJ/m^2), a conversion factor of 0.5 was multiplied by the daily incident solar radiation (Meek *et al*., 1984). To estimate the amount of daily intercepted PAR during each regrowth cycle, the linear regression between the fractional radiation interception estimated for the residual biomass (Section 6.1.2) and the measured fractional intercepted PAR at the end of each regrowth cycle in each plot was used. Daily solar radiation was then multiplied by daily fractional radiation interception. Total PAR intercepted for each regrowth cycle was therefore defined as the sum of calculated daily PAR in that regrowth cycle.

6.1.5 Measuring Leaf Area Index (LAI)

The Beer-Lambert law is regularly used to define the relationship between the proportion of light that penetrates a crop canopy and the leaf area index (e.g. Anderson, 1966; Brown, 1984; Brown and Parker, 1994; Maass *et al*., 1995):

Equation 6-2 $Q_i/RO = e^{-kLAI}$

In this equation Q_i is the irradiance under the crop canopy, RO is the irradiance above the crop canopy and k is the extinction coefficient.

Over 95 per cent of light interception results in maximum photosynthetic activity and hence maximum rate of growth (Peri *et al*., 2002). Thus, in the current study the critical LAI was defined for each grass species once the proportion of R/RO was ≥0.95.

Extinction coefficient (k)

Previous studies defined the extinction coefficient (k) as a constant number which is the slope of the relationship between Ln (R/Ro) and LAI and is only dependent on ELADP (Ellipsoidal Leaf Angle Distribution Parameter). Since k is related to the zenith angle and changes throughout the day and seasonally, the use of a constant k is not ideal. In this study, zenith angle data obtained from the sunscan were considered to calculate k values for each measurement. The extinction coefficient (k) in the current study was calculated based on Equation 6-3 derived by Campbell (1986).

Equation 6-3 $K(X,\theta) = \frac{\sqrt{X^2+\tan(\theta)^2}}{x+1.702(x+1.12)^{-0.708}}$

In this equation X is ELADP and θ is the zenith angle of the direct beam.

K values calculated from this equation were replaced in Equation 6-2 to calculate LAI. The LAI calculated by this method is then called THE adjusted LAI (LAIadj).

6.1.6 Calculating radiation use efficiency (RUE)

From 11/6/2015 to 10/07/2016 at Ladbrooks and from 8/7/2015 to 18/07/2016 at Ashley Dene, accumulated DM production in each destructive harvest was plotted against accumulated intercepted PAR for each species/N level plot. Radiation use efficiency (RUE) was then defined as the slope of the fitted linear regression. Because the amount of light intercepted by pasture residual was estimated at Ladbrooks, the linear regression was forced through the origin. However, due to the limitations at Ashley Dene (Section 6.1.3), estimation of residual biomass was not possible and could have been inaccurate. Therefore the linear regression lines was not forced through the origin at Ashley Dene.

6.1.7 Statistics

Genstat 16.1. was used to analyse the total amount of intercepted annual PAR (MJ/m^2), seasonal PAR intercepted by pastures, seasonal RUE and to calculate the slopes of linear regressions used to define annual RUE. In 2015/16, annual RUE was analysed using a strip-plot design with ±N strips as the rows and pasture species as columns.

Repeated measures analysis was used to compare seasonal PAR intercepted by pastures for each regrowth cycle.

6.2 Results

6.2.1 The quantity of annual PAR intercepted by pastures

Experiment 1 at Ladbrooks

From 16 June 2015 to 10 July 2016 at Ladbrooks, there was no interaction (P=0.148) between N and species for total intercepted PAR (Table 6-1). Mean total PAR intercepted was higher (P<0.001) for +N treatments. There was an indication (P=0.089) among species that the mean total intercepted PAR differed. Mean total PAR intercepted by tall fescue and cocksfoot was higher than brome. Perennial ryegrass was intermediate (Table 6-1).

Table 6-1 Total intercepted PAR (MJ PAR m^{-2} yr^{-1}) for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue monocultures at Ladbrooks, Canterbury, New Zealand. Results are based on data collected from 11/6/2015 to 10/7/2016.

	Nitrogen level (N)		
Species (S)	+N	−N	Mean
Brome	2074	1576	1825
Cocksfoot	2111	1721	1916
P. ryegrass	2183	1590	1887
Tall fescue	2123	1752	1938
Mean	2123	1660	
S	0.089		
N	<.001		
S*N	0.148		
LSD (S)	88.6		
LSD (N)	20.9		
LSD (S*N)	-		

Note: S, N and S*N show the P values for species, nitrogen and species*N interactions.

Experiment 2 at Ashley Dene

From 8/7/2015 to 18/7/2016, there was an interaction (P<0.05) between N and species for total intercepted PAR.

Nitrogen application increased (P<0.001) total intercepted PAR for brome and cocksfoot but not perennial ryegrass and tall fescue (Table 6-2). Mean total PAR intercepted by cocksfoot pastures (1049 ± 15.3 MJ/m^2 yr^{-1}) was higher (P<0.001) than other species. Total PAR intercepted by +N cocksfoot pasture was 50-54% higher than other +N pastures. PAR intercepted by −N cocksfoot was also 42-47% higher than other −N species (Table 6-2).

Table 6-2 Total intercepted PAR (MJ PAR m^{-2} yr^{-1}) for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue at Ashley Dene, Canterbury, New Zealand. Data are based on data collected from 8/7/2015 to 18/7/2016.

	Nitrogen level (N)		
Species (S)	+N	−N	Mean
Brome	600	503	552 b
Cocksfoot	1178	919	1049 a
P. ryegrass	591	536	564 b
Tall fescue	544	500	515 b
Mean	624	560	
S	<0.001		
N	<0.001		
S*N	0.019		
LSD (S)	77.9		
LSD (N)	47.2		
LSD (S*N)	96.3		

Note: S, N and S*N show the P values for species, nitrogen and species*N interactions. Means within a column with different letters are significantly different (LSD at the α≤0.05 level).

6.2.2 Seasonal PAR intercepted by pastures

Experiment 1 at Ladbrooks

There was no interaction (P=0.105) between species and N for total intercepted PAR (MJ m^{-2}). Based on repeated measures analysis results, species showed difference (P<0.05) intercepted PAR at different times of the year. The quantity of intercepted PAR (MJ/m^2) by the pastures differed among species in five of the nine regrowth cycles (Figure 6-2). On 14/10/2015, mean intercepted PAR by brome and perennial ryegrass pastures was lower (P<0.05) than the other species. In summer (9/1/2016), mean intercepted PAR by perennial ryegrass pastures was less than the three other species. On 5/2/2016, intercepted PAR by cocksfoot was higher (P<0.05) than the other species. Repeated measures analysis also showed an interaction between N and time (P<0.001) from 10/11/2015 to 10/7/2016. The first N was applied on 18/9/2015 at Ladbrooks. Before application of N fertiliser, there was no difference (P=0.399) between ±N treatments for intercepted PAR on 11/9/2015. Once N fertiliser was applied, intercepted PAR by +N pastures was always higher (P<0.001) than −N pastures (Figure 6-2).

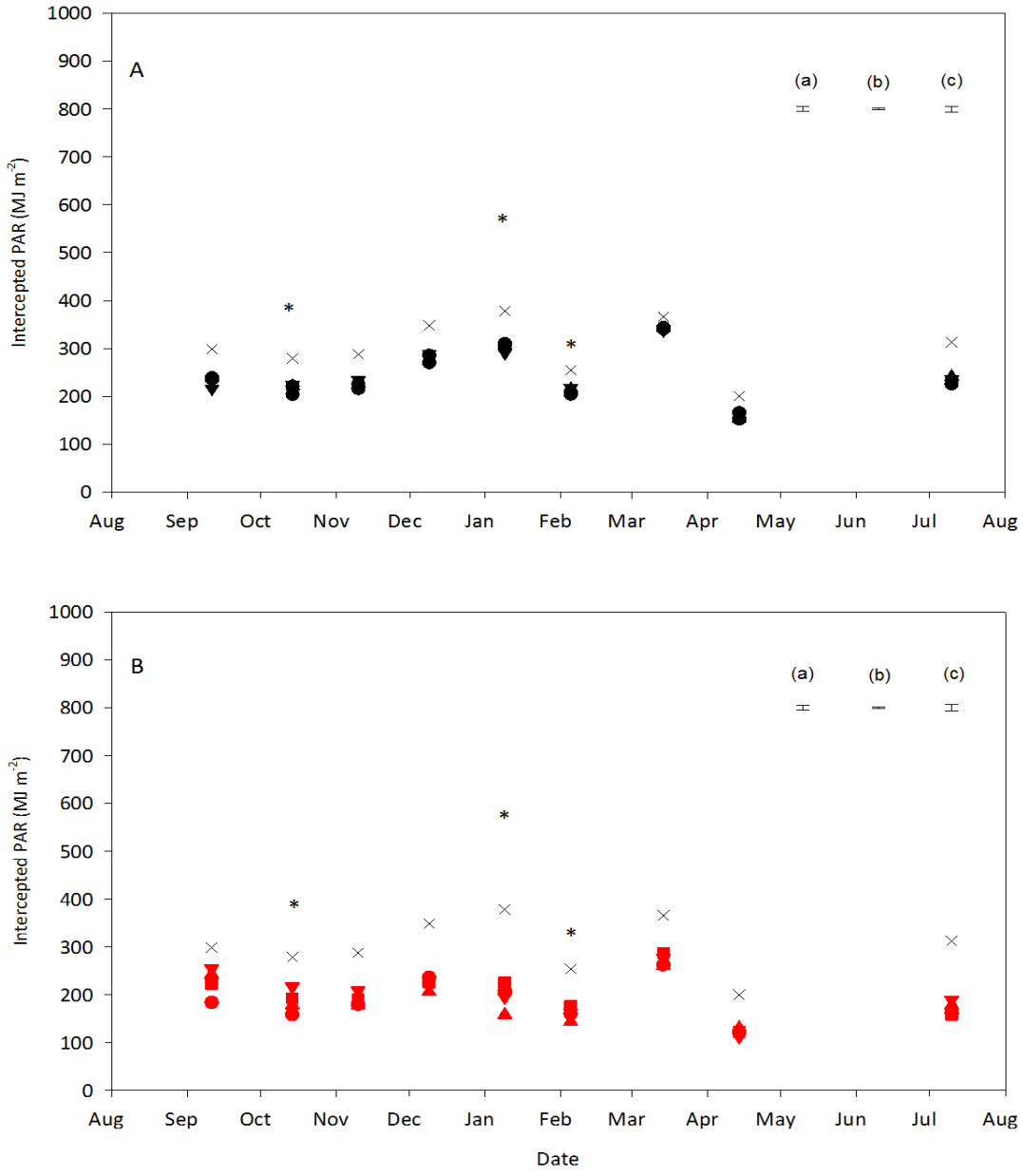


Figure 6-2 Quantity of photosynthetically active radiation intercepted (MJ m^{-2}) by +N (A) and −N (B), brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) monocultures at Ladbrooks, Canterbury, New Zealand throughout individual regrowth cycle in 2015/16. The crosses (×), represent total incident PAR receipts for each regrowth cycle. Error bars are maximum SEM for (a) species, (b) N and (c) species*N interactions. Asterisks show where differences were observed.

Experiment 2 at Ashley Dene

Species differences in total intercepted PAR (MJ m^{-2}) occurred seasonally. The quantity of PAR intercepted by the cocksfoot pastures was higher (P<.001) than other species in three of the five regrowth cycles. The mean intercepted PAR by cocksfoot pastures was higher than other species on 27/10/2015, 20/1/2016 and 21/4/2016 (Figure 6-3). There was also an interaction (P<0.05) between

N and time for intercepted PAR. The results showed that on two occasions on 20/1/2016 and 21/4/2016, N increased total PAR intercepted by cocksfoot.

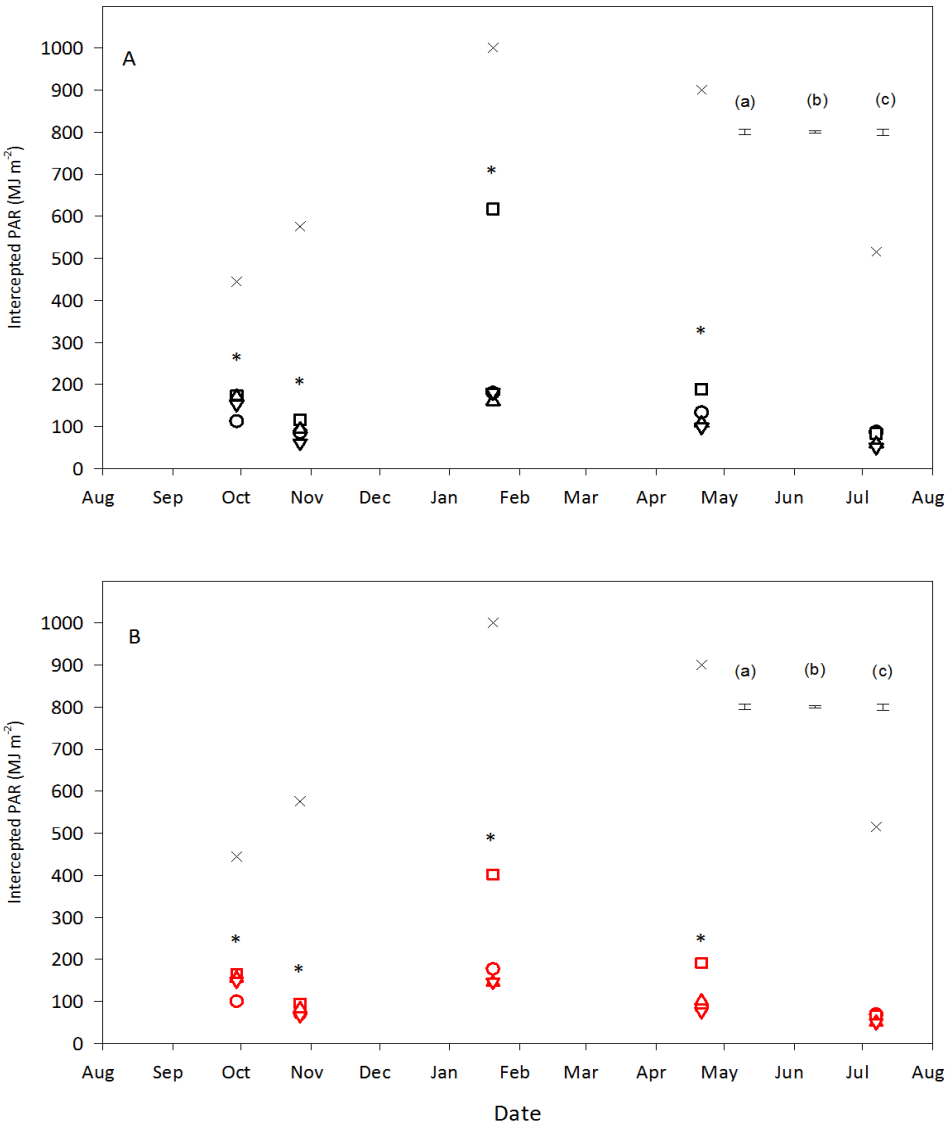


Figure 6-3 Quantity of photosynthetically active radiation intercepted (MJ m^{-2}) by +N (A) and −N (B) for brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) monocultures at Ashley Dene, Canterbury, New Zealand throughout individual regrowth in 2015/16. The crosses (×), represent total incident PAR receipts for each regrowth cycle. Error bars are maximum SEM for (a) species, (b) N and (c) species*N interactions. Asterisks show where differences were observed.

6.2.3 Radiation use efficiency (RUE)

Experiment 1 at Ladbrooks

There was a linear relationship between accumulated intercepted PAR and accumulated DM with a mean R^2 of 0.98 for all species and N levels (Figure 6-4). From 11/6/2015 to 10/07/2016, annual RUE

of the +N pastures was higher (P<0.001) than −N pastures. The average annual RUE was not different (P=0.315) among species (Table 6-3).

Table 6-3 Annual radiation use efficiency (g DM/MJ PAR) of brome, cocksfoot, perennial ryegrass and tall fescue monocultures at Ladbrooks, Canterbury, New Zealand. Data are based on data collected from 11/6/2015 to 10/7/2016. Regressions used to calculate RUE are presented in Figure 6-4.

	N level (N)		
Species (S)	+N	−N	Mean
Brome	1.03	0.618	0.824
Cocksfoot	1.07	0.701	0.886
P. ryegrass	1.14	0.740	0.940
Tall fescue	1.00	0.628	0.814
Mean	1.06	0.671	
S	0.315		
N	0.001		
S*N	0.502		
LSD (S)	-		
LSD (N)	0.192		
LSD (S*N)	-		

Note: S, N and S*N show the P values for species, N and species*N interactions.

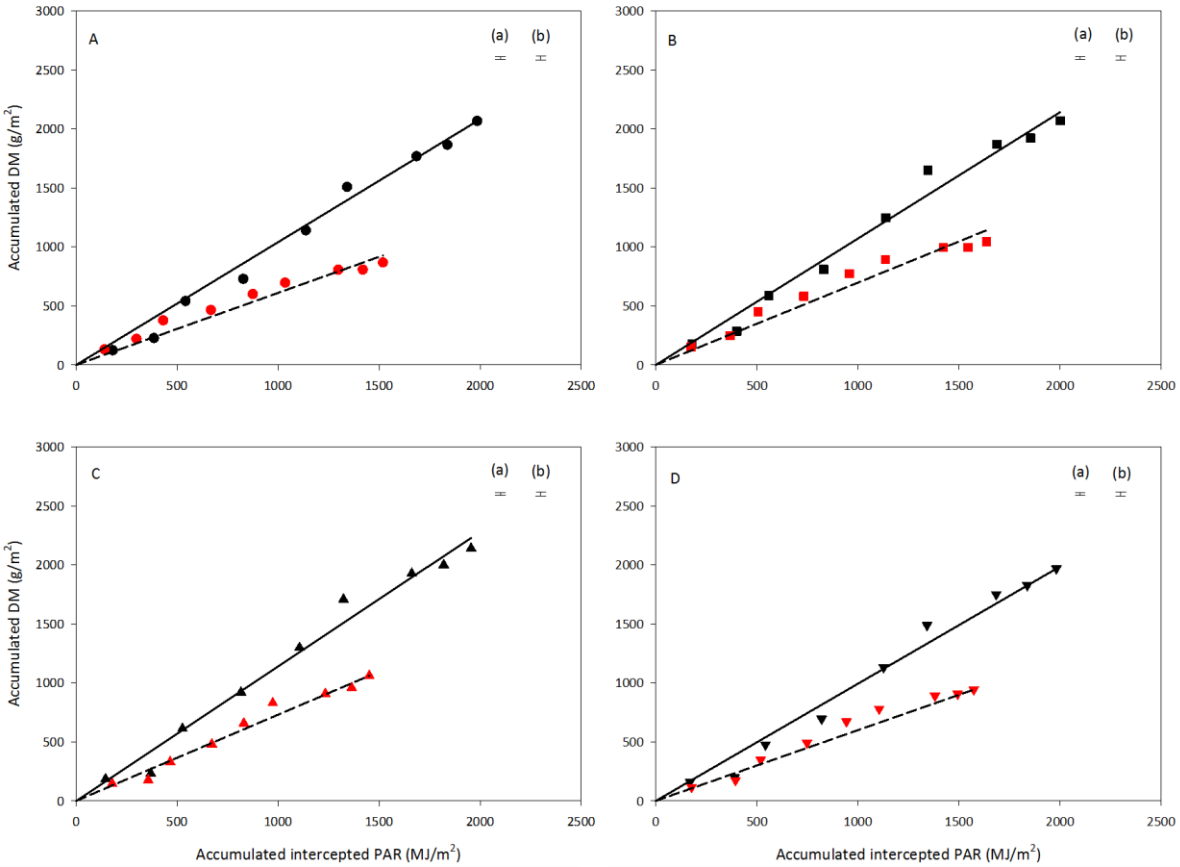


Figure 6-4 Accumulated yield (g DM/m^2) against accumulated intercepted photosynthetically active radiation (MJ/m^2) by +N (black) and −N (red) monocultures of (A) brome (●,●), (B) cocksfoot (■,■), (C) perennial ryegrass (▲,▲) and (D) tall fescue (▼,▼) at Ladbrooks, Canterbury, New Zealand. Data are based on data collected from 11/6/2015 to 10/7/2016. Linear regression equation details fitted to +N (—) and −N (– – –) are reported in Table 6-4. Error bars are maximum SEMs for (a) species and (b) N.

Table 6-4 Regression equations for accumulated yield (g DM/m^2) against accumulated intercepted PAR (MJ/m^2) by + and −N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue at Ladbrooks, Canterbury, New Zealand.

Species (S)	Equation (Y=)	SE Coef (x)	R^2
N level (N)		+N	
Brome	1.03x	0.044	0.97
Cocksfoot	1.07x	0.057	0.97
P. ryegrass	1.14x	0.056	0.98
Tall fescue	1.00x	0.053	0.96
N level (N)		−N	
Brome	0.618x	0.0279	0.97
Cocksfoot	0.701x	0.0390	0.98
P. ryegrass	0.740x	0.0478	0.96
Tall fescue	0.629x	0.0442	0.97

Experiment 2 at Ashley Dene

From 8/7/2015 to 18/07/2016, N did not affect (P=0.370) RUE of the pastures at Ashley Dene (Figure 6-5). Mean RUE was 0.547 ± 0.0269 g DM/MJ PAR and was not different among species (Table 6-5). There was a strong linear relationship (R^2≥97%) between accumulated yield (g DM/m^2) and accumulated intercepted PAR (MJ/m^2) for brome, perennial ryegrass and tall fescue. However, the linear relationship was not as strong as the other species both for +N (R^2=91%) and −N (R^2=94%) cocksfoot. Based on the results in Figure 6-5, the linear relationships between accumulated yield and accumulated intercepted PAR for cocksfoot pastures at Ashley Dene can be broken to two phases. Phase one is based on the first two measurements taken in September and October 2015. Phase two, starts from January 2016 and ends on July 2016 in which the slope of the linear regression (RUE) is higher than phase one. Therefore, using a single value of RUE, which is calculated as the slope of linear regression fitted between accumulated yield (g DM/m^2) and accumulated intercepted PAR for cocksfoot pastures might be inappropriate at Ashley Dene.

Table 6-5 Annual radiation use efficiency (g DM/MJ PAR) of brome, cocksfoot, perennial ryegrass and tall fescue monocultures at Ashley Dene, Canterbury, New Zealand. Data are based on data collected from 8/7/2015 to 18/07/2016. Regressions used to calculate RUE are presented in Table 6-6.

	Nitrogen level (N)		
Species (S)	+N	−N	Mean
Brome	0.510	0.511	0.511
Cocksfoot	0.522	0.524	0.523
P. ryegrass	0.630	0.681	0.656
Tall fescue	0.439	0.559	0.499
Mean	0.525	0.569	
S	0.075		
N	0.370		
S*N	-		
LSD (S)	-		
LSD (N)	-		
LSD (S*N)	-		

Note: S, N and S*N show the P values for species and nitrogen. Means within a column with different letters are significantly different (LSD at the α≤0.05 level).

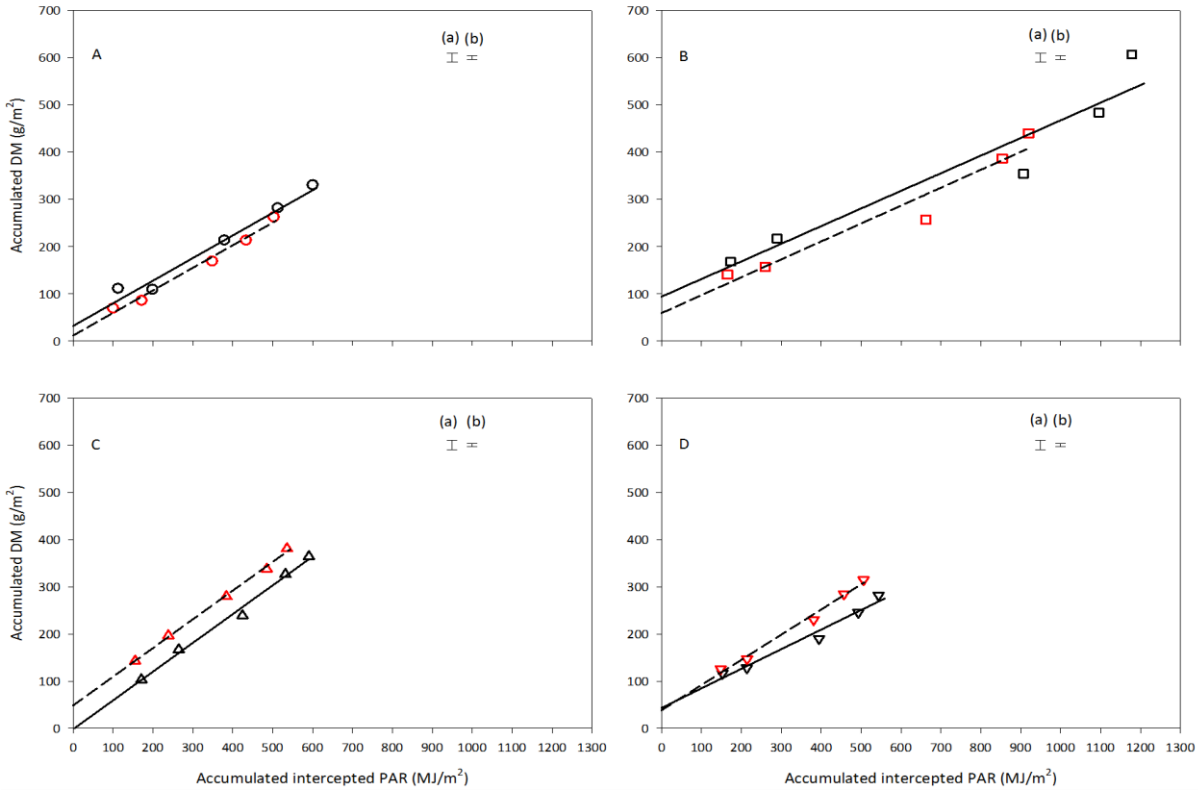


Figure 6-5 Accumulated dry matter (DM) (g/m^2) against accumulated intercepted photosynthetically active radiation (MJ/m^2) by +N (black) and −N (red) monocultures of (A) brome (○,○), (B) cocksfoot (□,□), (C) perennial ryegrass (△,△), and (D) tall fescue (▽,▽) at Ashley Dene, Canterbury, New Zealand. Data are based on data collected from 8/7/2015 to 18/7/2016. Linear regressions equations details fitted to +N (—) and −N (– – –) are reported in Table 6-6. Error bars are maximum SEMs for (a) species and (b) N.

Table 6-6 Regression equations for accumulated yield (g DM/m^2) against accumulated intercepted PAR (MJ/m^2) by + and −N monocultures of brome, cocksfoot, perennial ryegrass and tall fescue at Ashley Dene, Canterbury, New Zealand.

Species (S)	Equation (Y=)	SE Coef (x)	SE Coef (constant)	R^2
N level (N)		+N		
Brome	0.510x+37	0.0444	17.9	0.98
Cocksfoot	0.522x+94	0.0681	57.2	0.91
P. ryegrass	0.630x-2.0	0.0333	14.2	0.99
Tall fescue	0.439x+43.1	0.04047	15.8	0.97
N level (N)		−N		
Brome	0.511x+11.5	0.0327	11.3	0.99
Cocksfoot	0.524x+59.2	0.0546	35.4	0.94
P. ryegrass	0.681x+48.3	0.0173	48.3	0.99
Tall fescue	0.559x-38.3	0.0316	11.6	0.99

6.2.4 Seasonal RUE

Experiment 1 at Ladbrooks

Except from 11/9/2015, N increased ($P<0.05$) RUE in the rest of the year. Species treatments were different on three occasions on 14/10/2015, 10/11/2015 and 14/03/2016 (Figure 6-6). For example during the winter (11/9/2015 to 14/10/2015) the average RUE for brome was higher ($P<0.001$) than other species which reflects its different growth rate in winter.

Figure 6-6 shows that, increasing the average temperature from September 2015 to February 2016 caused an increase in RUE. The exception was in the middle of March, when the lack of soil moisture (Chapter 5) restricted growth.

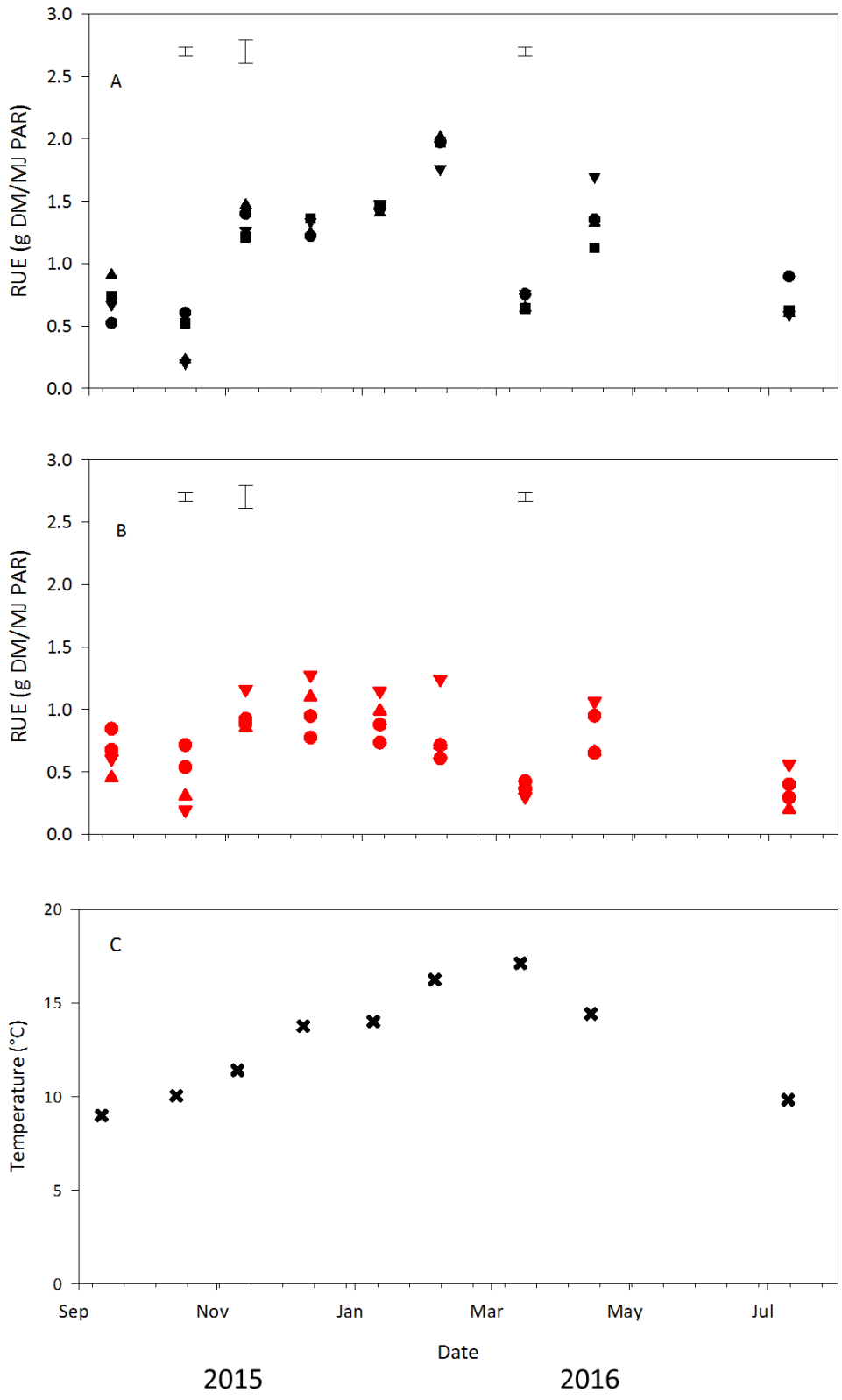


Figure 6-6 Radiation use efficiency (RUE) for individual regrowth cycles by +N (A) and −N (B) monocultures of brome (●,●), cocksfoot (■,■), perennial ryegrass (▲,▲) and tall fescue (▼,▼) and mean regrowth cycle temperature (C) at Ladbrooks, Canterbury, New Zealand. Error bars show the LSDs when there was a difference among species.

Experiment 2 at Ashley Dene

RUE differed among species on 27/10/2015. At that time, the average RUE for perennial ryegrass and tall fescue pastures (0.680 ± 0.0913 g DM/MJ PAR) was higher (P<0.05) than cocksfoot (0.267 g DM/MJ PAR) but not different from brome (Figure 6-7). Since Ashley Dene was mostly under moisture stress (Chapter 5), it is hard to find a clear relationship between temperature and RUE as at Ladbrooks.

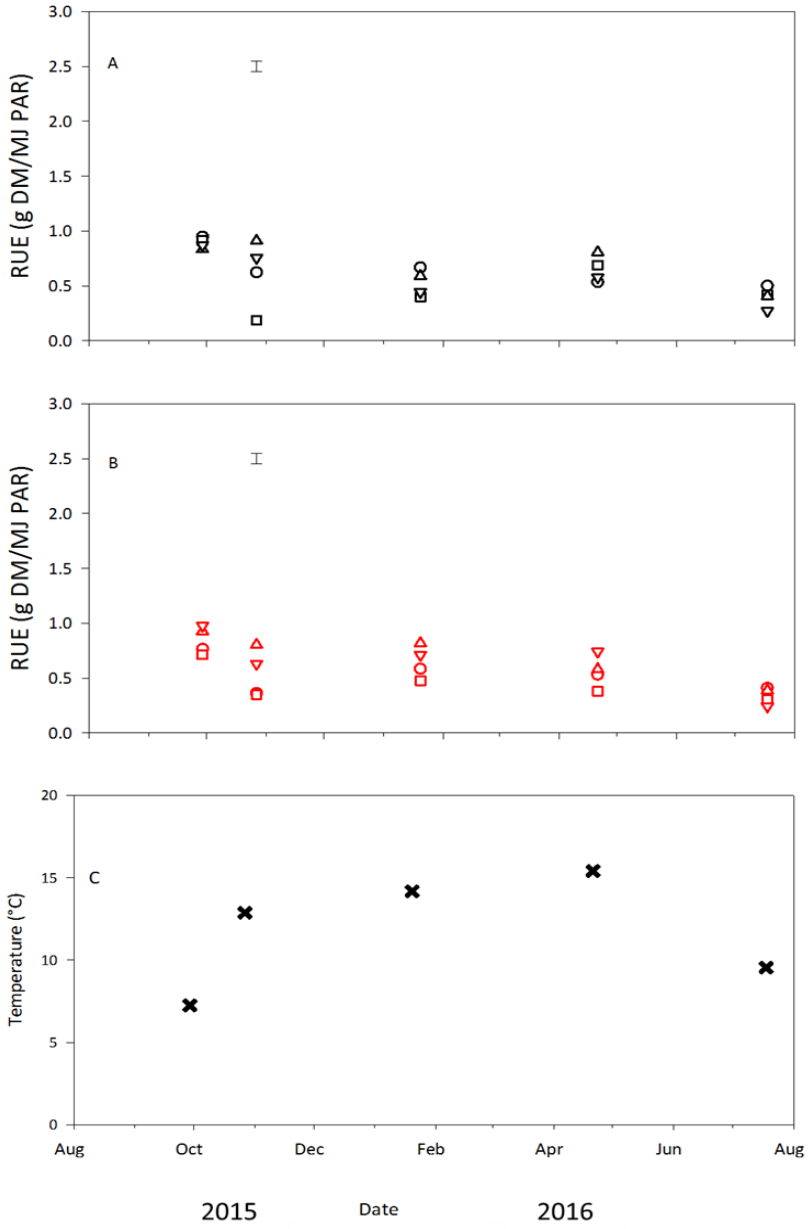


Figure 6-7 Radiation use efficiency (RUE) for individual regrowth cycles by +N (A) and −N (B) monocultures of brome (○,○), cocksfoot (□,□), perennial ryegrass (△,△), and tall fescue (▽,▽) and mean regrowth cycle temperature (C) at Ashley Dene, Canterbury, New Zealand. Error bars show the LSDs once there was a difference among species.

6.2.5 Leaf area index (LAI)

Figure 6-8 shows the relationship between intercepted PAR (R/RO) and adjusted leaf area index (LAIadj) for all treatments at different plots from 11/6/2015 to 10/7/2016 at Ladbrooks and from 8/7/2015 to 18/07/2016 at Ashley Dene. The average critical leaf area index (LAIadj) was 4.05.

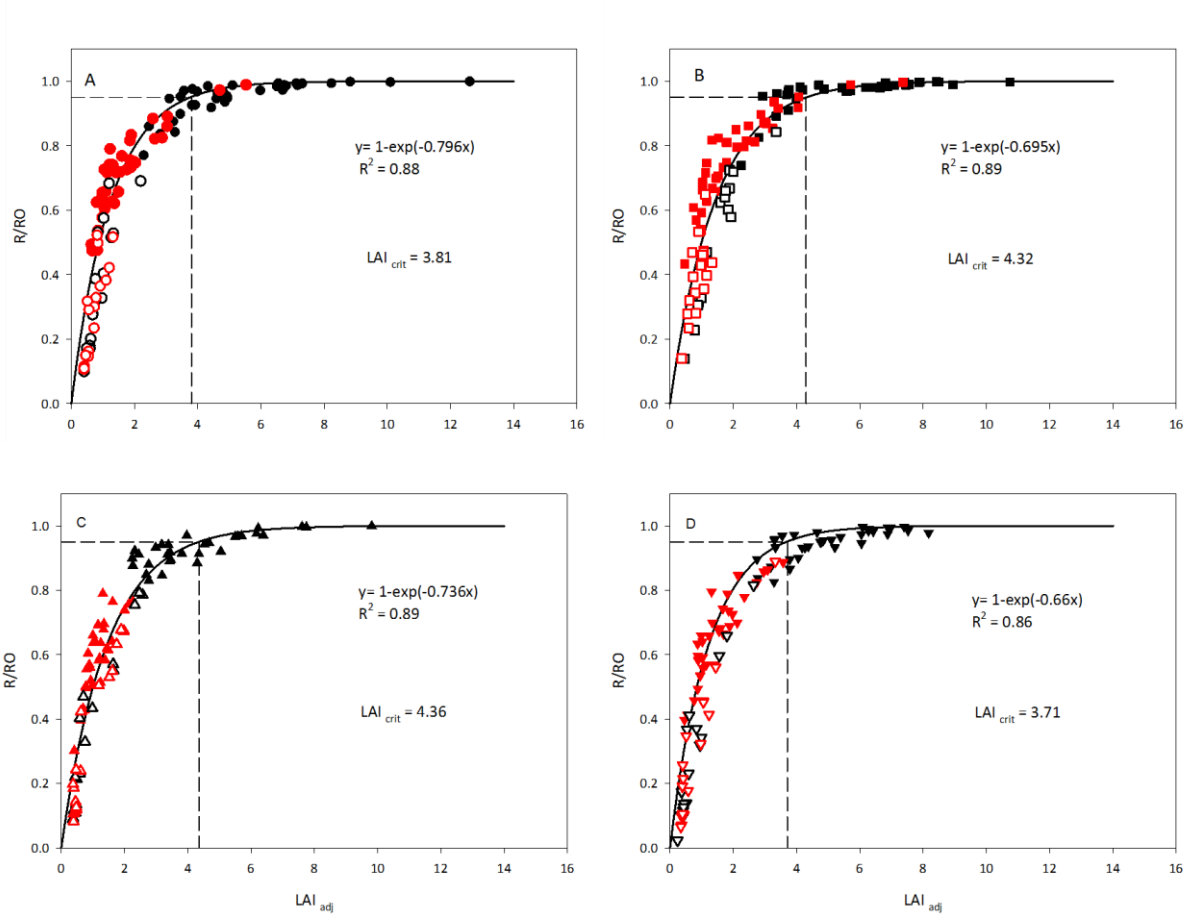


Figure 6-8 Relationship between adjusted Sunscan leaf area index (LAIadj) and fractional intercepted PAR (R/Ro) by +N (black) and −N (red) monocultures of (A) brome, (B) cocksfoot, (C) perennial ryegrass and (D) tall fescue at Ladbrooks (filled symbols) and Ashley Dene (open symbols), Canterbury, New Zealand. The critical LAI (LAIcrit) when 95% incident of PAR was intercepted.

6.2.6 Seasonal LAI

Experiment 1 at Ladbrooks

Repeated measures analysis showed that there was an interaction ($P<0.01$) between time and species. This was because the average LAIadj for cocksfoot was higher than other species on 14/9/2015, 10/10/2015 and 14/3/2016. The average LAIadj for brome and cocksfoot was higher than other species on 9/1/2016. On 5/2/2016 the average LAIadj for cocksfoot was higher than tall fescue but not different from the other three species. On 10/7/2016, perennial ryegrass and then tall fescue had the lowest LAIadj (Figure 6-9).

There was also an interaction ($P<0.01$) between time and N. This was because applying N fertiliser had lower impact on the average LAIadj of the pastures on 9/12/2015 and in summer on 14/3/2016 and 14/4/2016 than at other measurements time. There was no interaction ($P=0.06$) between species and N.

Except from 14/9/2015 (before N application), on the other eight measurement dates, N caused an increase ($P<0.001$) in LAIadj from 13/10/2015 to 10/7/2016 at Ladbrook. During the same period, on the five measurement dates the average LAIadj for cocksfoot was higher ($P<0.01$) than perennial ryegrass. LAIadj for −N pastures was always under the critical LAI (LAIcrit) except from 14/9/2015 (Figure 6-9). This might be due to the N background still present at the site in the rotation that started from 16/7/2015 and ended on 14/9/2015. All species showed the lowest LAIadj during summer from 14/3/2016 to 14/4/2016.

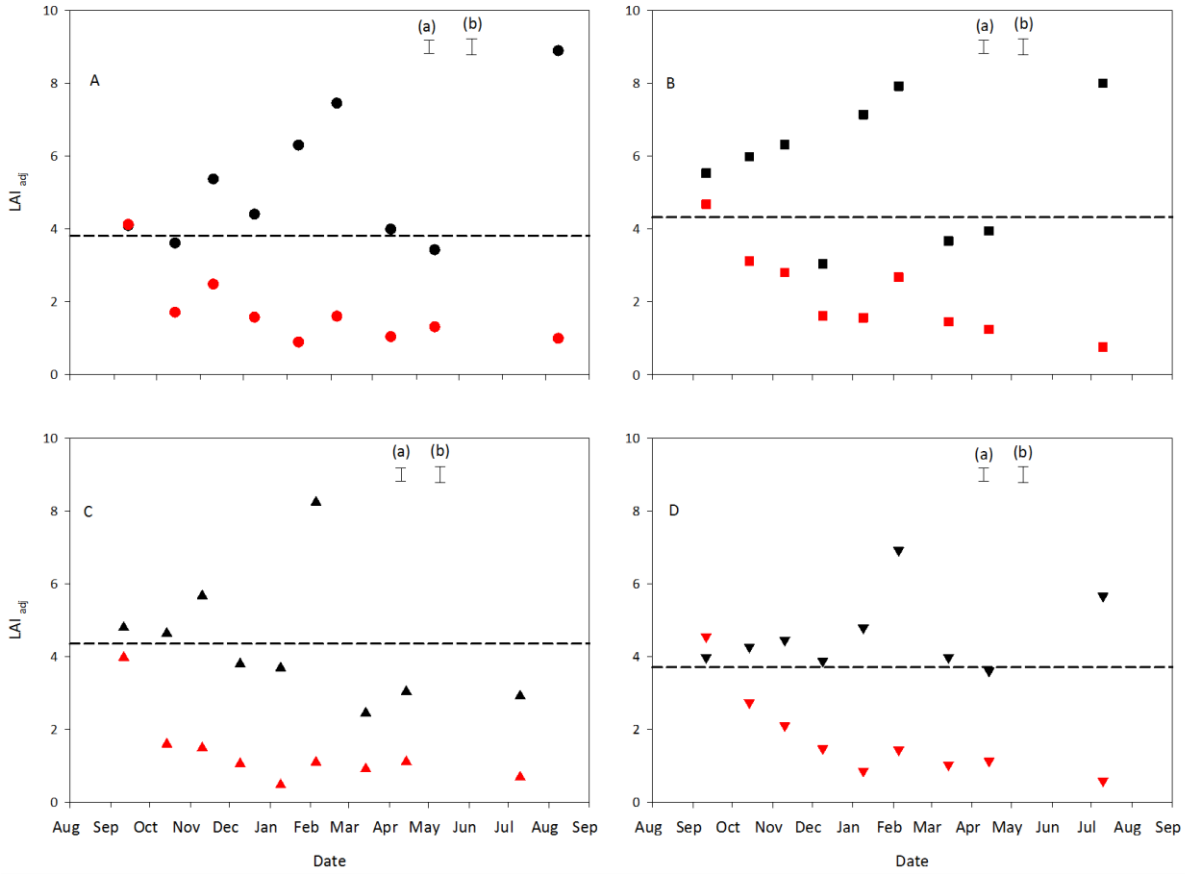


Figure 6-9 Adjusted leaf area index (LAIadj) at the end of individual regrowth cycles by +N (black) and −N (red) of monocultures of (A) brome (●,●), (B) cocksfoot (■,■), (C) perennial ryegrass (▲,▲) and (D) tall fescue (▼,▼) pastures in 2015/16 at Ladbrooks, Canterbury, New Zealand. Error bars are maximum SEM for (a) species and (b) N. Dashed lines show the critical LAI (LAIcrit) for each species.

Experiment 2 at Ashley Dene

Repeated measures analysis showed that for LAI there was an interaction (P<0.001) between species and time. Cocksfoot showed the highest LAI on 27/10/2015, 20/1/2016 and 21/4/2016. On 7/7/2016, the average LAI for brome and cocksfoot was higher than other species. There was also an interaction (P<0.001) between species and N. The average LAI for +N brome and cocksfoot was higher than their control (−N). The average LAI for +N perennial ryegrass and tall fescue were not different compared with their control (−N) (Figure 6-10).

No interaction (P=0.145) was found between time and N.

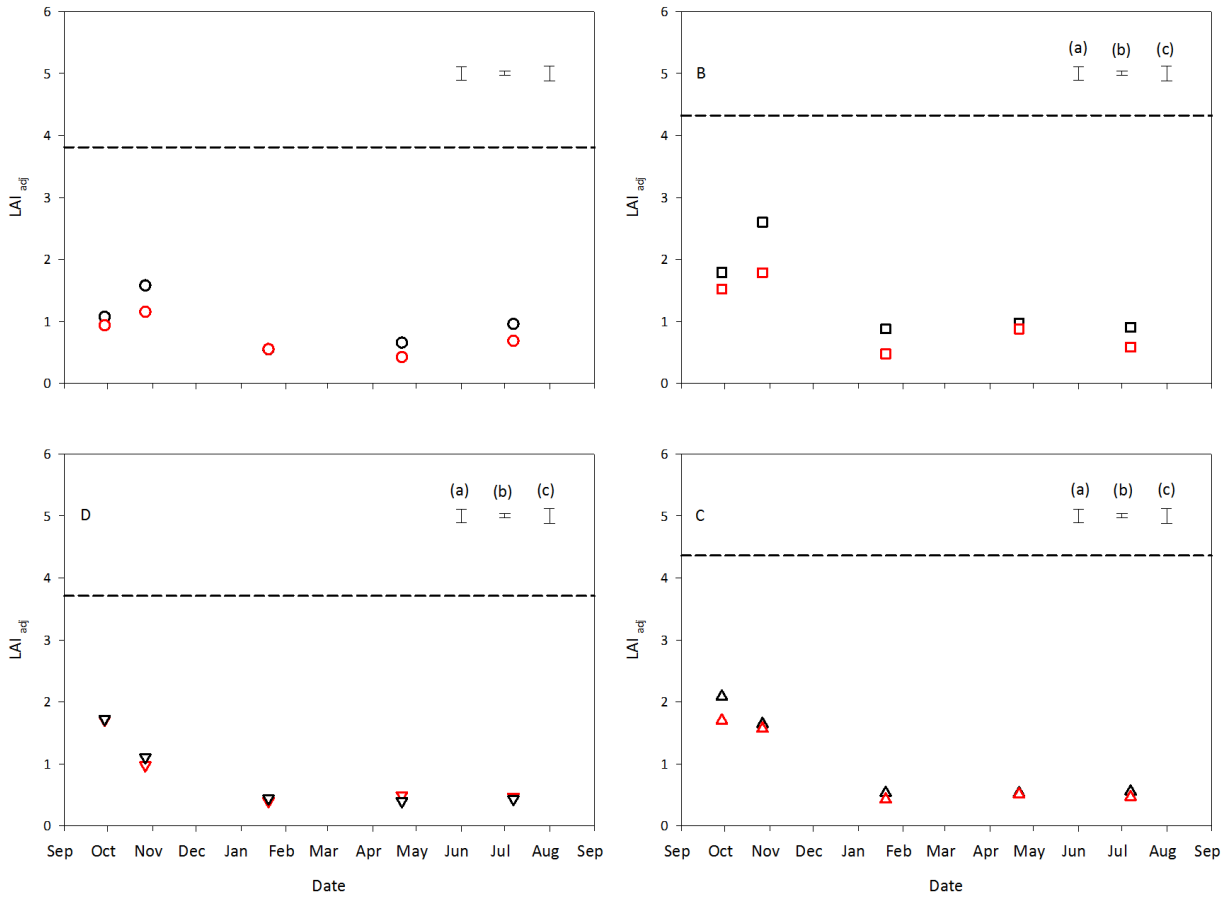


Figure 6-10 Adjusted leaf area index (LAIadj) at the end of individual regrowth cycles by +N (black) and −N (red) of monocultures of (A) brome (○,○) , (B) cocksfoot (□,□), (C) perennial ryegrass (△,△) and (D) tall fescue (▽,▽) in 2015/16 at Ashley Dene, Canterbury, New Zealand. Error bars are maximum SEM for (a) species, (b) N and (c) species*N interactions. Dashed lines show the critical LAI (LAIcrit) for each species.

6.3 Discussion

Radiation interception and RUE are the major factors that influence yield formation (Equation 2-1). In this chapter canopy expansion (quantified by LAI) and light interception were quantified (Objective 8, Section 1.4). Objective 9 was to investigate whether the yield differences resulted from differences in radiation interception and/or RUE.

In this section the combined effects of different levels of moisture and N on R/RO and RUE are discussed. To do that, first the major limitations for using Sunscan plant canopy analyser (Delta-T Devices Ltd., Burwell, Cambridge, England) in measuring light intercepted by pastures is considered.

6.3.1 How did different levels of moisture affect LAI, PAR and RUE by +N pastures?

Severe moisture stress at Ashley Dene, caused a reduction in LAIadj below the critical LAI all through the year (Section 6.2.6). In +N pastures at Ladbrooks also, LAIadj decreased below critical in March and April 2016, once actual soil moisture deficit exceeded 200 mm.

Under non limiting N, the main reason for lower DM production of brome, cocksfoot, perennial ryegrass and tall fescue at Ashley Dene compared with Ladbrooks were the lower PAR intercepted by all grasses as well as the lower RUE at Ashley Dene. The most important difference between the two experimental sites was the different soil types which had a higher PAWC for Ladbrooks compared with Ashley Dene (Section 4.5). Previous studies (Section 2.3.1) showed that, under moisture stress, decreased leaf WP results in a progressive drop in cell turgor pressure (Section 2.3.1). This supports the conclusion that, at Ashley Dene, reduction in cell expansion caused the formation of smaller leaves (Hsiao, 1973; Hay and Walker, 1989) and therefore less light was intercepted because LAI was always below critical (95% interception) (Johns, 1978). Smaller leaves would also reduce the available area for gas exchange (Johns, 1978). Therefore, formation of smaller leaves at Ashley Dene was the major reason for decreased PAR intercepted by the canopy.

Where soil moisture was more limited at Ashley Dene, there was a 70% reduction in total PAR intercepted by +N brome, perennial ryegrass and tall fescue compared with Ladbrooks. Comparing RUE between the two sites shows that, annual RUE at Ashley Dene was 35-45% of that at Ladbrooks. Hsiao (1973) also reported that, primarily, effects of moisture stress on cell expansion and division will have a greater effect on DM production than changes in RUE.

As described in Section 2.1, yield formation is a function of incident PAR, the fraction of PAR intercepted by the canopy (R/R0) and RUE. Total intercepted PAR for brome, perennial ryegrass and tall fescue at Ashley Dene was 30% of that at Ladbrooks. The combined effect of a 70% reduction in total intercepted PAR and 35-45% of lower annual RUE by +N brome, perennial ryegrass and tall fescue

caused an almost 15% of DM production for the mentioned species at Ashley Dene compared with Ladbrooks. Therefore, based on Equation 2-1, in 2015/16, DM production for brome, perennial ryegrass and tall fescue at Ashley Dene was expected to be 11% to 14% of that at Ladbrooks once N was not limited. This is similar to the results presented in Chapter 4, showed an average of 14% of Ladbrooks DM at Ashley Dene for +N pastures (Section 4.11.4). For +N cocksfoot, total intercepted PAR at Ashley Dene was 44% of that at Ladbrooks, and RUE was 56% of that at Ladbrooks. This should have resulted in a 24% of total DM of that at Ladbrooks, for cocksfoot at Ashley Dene (Sections 4.11.4, 6.2.1 and 6.2.3). However, total DM of +N cocksfoot at Ashley Dene was 30% of that at Ladbrooks. Therefore, it is likely that this is due to the underestimation of RUE for cocksfoot pastures which occurred between January to July 2016 at Ashley Dene (Section 6.2.3).

6.3.2 How did the combined effects of moisture and nitrogen levels affect LAI, PAR and RUE of the pastures?

Objective 9 was to investigate whether the yield differences between sites, N levels and among species (at Ashley Dene) resulted from the differences in radiation interception and/or RUE.

Based on the results from Ladbrooks, N application caused ~40% increase in RUE and 22% rise in total PAR intercepted. Mills (2006) found a similar intercepted PAR (2250 MJ PAR m^{-2}) by irrigated +N 'Wana' cocksfoot in 2004/5 when Delta-T Devices Ltd (Burwell, Cambridge, U.K) equipment was used to measure the intercepted light. They found a potential total DM production of 21.9 t DM ha^{-1} from irrigated +N 'Wana' cocksfoot which was comparable to the total DM of 19.8 t DM ha^{-1} for +N pastures at Ladbrooks in the current study. Under the same conditions, mean total intercepted PAR by −N pastures at Ladbrooks was 19% lower than irrigated −N 'Wana' cocksfoot in 2004/5 reported by Mills (2006). However, total DM production of −N pastures at Ladbrooks (9.2 t DM ha^{-1}) was similar to the total DM of irrigated −N 'Wana' cocksfoot (9.6 t DM ha^{-1}). There was 23% of dead material and weeds in yield of an eight years old 'Wana' cocksfoot pasture (18% dead material + 5% weeds) reported by Mills (2006). Since the device dose not detect the difference between dead materials (Section 6.1.3), weeds and green grasses, total PAR intercepted by 'Wana' cocksfoot might have been overestimated. Mills (2006) concluded that overestimating PAR interception by −N pastures probably reflect the inherent difficulty associated with measuring light interception in perennial pastures. This is due to a residual biomass that remains after cutting/grazing and additional limitations which include the inability of the equipment to differentiate between green and non-green pasture components. Overestimation of total PAR intercepted by −N pastures in Mills (2006) study, resulted in finding a similar PAR intercepted by irrigated −N and +N pastures with an almost three times higher RUE for +N cocksfoot. So, they concluded that the difference between DM production of irrigated + and −N cocksfoot was only due to a higher RUE for +N cocksfoot. Total dead material in pastures at Ladbrooks

was minimal (<3%) in the current study (Section 4.11.6). Therefore, at Ladbrooks, the lower DM production of −N pastures compared with +N pastures was due to the both higher intercepted PAR and higher RUE in +N pastures compared with the control. In −N pastures, the amount of chlorophyll would likely be decreased and lower PAR intercepted by leaf surfaces is converted to chemical energy per unit area.

Mean total intercepted PAR by cocksfoot pastures was higher than other species at Ashley Dene. Total intercepted PAR by −N cocksfoot, was 55-58% and for +N was 46-51% higher than the other pasture grasses with the same level of N. The number of tillers, leaf appearance and also leaf elongation were not measured in this study. However, it is likely that more intercepted PAR by cocksfoot pastures at Ashley Dene was due to its higher tiller production compared with the other grasses. Subsequently, the major reason for increased DM production was due to a greater surface area for gas exchange and photosynthesis. Norris (1982) showed that moisture stressed cocksfoot produced 3.0 g m^2 d^{-1} compared with 4.0 g m^2 d^{-1} for irrigated cocksfoot. The leaf extension rate of both species declined by 40% compared with irrigated plants but dryland cocksfoot had 19.2 tillers/plant compared with ryegrass which had 9.6 tillers/plant. Consequently, cocksfoot probably intercepted more PAR than ryegrass under dryland conditions.

Mills (2006) showed that, as the ASMD increased under dryland conditions, reduction in the number of tillers was 21 tillers/m^2/mm for +N compared with 13 tillers/m^2/mm for −N cocksfoot. Further work is required to gather information on the tiller numbers.

More than 50% of soluble N is directly related to the formation of photosynthetic apparatus and also N stimulates tiller production (Baker and Younger, 1987). Nitrogen is necessary in chlorophyll formation, the majority of which exists within mesophyll cells (MacAdam *et al*., 1989). These are the major factors for PAR interception and conversion of light energy to the required chemical energy which drives photosynthesis (Campbell, 1996). Therefore, increased photosynthetic efficiency (RUE) combined with increased PAR intercepted by a canopy due to more leaves per unit area would contribute to an increase in DM production under no N limitation at Ladbrooks. At Ashley Dene, where severe moisture stress limited extraction of N fertiliser by the pastures, N application did not affect the amount of intercepted PAR (and RUE) by perennial ryegrass or tall fescue pastures. However, application of N resulted in 17% and 25% increase in total PAR intercepted by +N brome and cocksfoot but caused no changes in RUE. Therefore, the results of the current study at Ashley Dene show that, under a severe moisture stress, N application might have increased tiller production and/or formation of larger cells for brome and cocksfoot but not increased the amount of chlorophyll as RUE was unchanged. Therefore, it can be concluded that, there might be different mechanisms for +N brome and cocksfoot under severe moisture stress which caused them to intercept more light but have no

change in RUE. The inherent difficulty related to measuring light interception due to presence of stones at the soil surface at Ashley Dene may also cause inaccuracy in measurements.

Under severe moisture stress at Ashley Dene, interception of more PAR by cocksfoot compared with other grasses used in this study in late spring and summer (on 20/1/2016 and 20/4/2016) meant that, under summer dry conditions, only 85 mm of rainfall on January 2016 and <50 mm (from 20/1/2016 to 20/4/2016) enabled +N cocksfoot to intercept higher PAR which caused an increase in its DM production.

6.4 Conclusions

This chapter, dealt with Objectives 8 and 9 (Section 1.4). Based on the results, the following conclusions can be made:

- At Ladbrooks, LAI for +N was higher than −N pastures on eight occasions.
- LAIadj for cocksfoot was higher than the other species on 27/10/2015, 20/1/2016 and 21/4/2016 at Ashley Dene. The average LAIadj for +N brome and cocksfoot was higher than their control (−N).
- Nitrogen caused ~40% increase in RUE and 22% rise in total PAR intercepted at Ladbrooks. Therefore, the lower DM production of −N compared with +N pastures at Ladbrooks was due to the both higher intercepted PAR and also a higher RUE in +N pastures compared with the control (−N).
- Higher production of +N cocksfoot compared with other species at Ashley Dene was mainly due to more PAR intercepted by cocksfoot.
- At Ashley Dene, mean RUE was 0.547 ± 0.0269 g DM/MJ PAR and was not different among species. Annual RUE at Ashley Dene was 35-45% of that at Ladbrooks.

Chapter 7 General discussion

7.1 Introduction

The main aim of this research (Section 1.4) was to understand, the effects of different levels of moisture and N on dryland monocultures of brome, cocksfoot, perennial ryegrass and tall fescue growth under summer dry conditions. The underlying assumption was that, improved understanding of limitations and benefits of each species will aid agronomic advice to increase production of dryland farming systems. To investigate the effect of different moisture levels on production, two field experiments in the same climate, but on two sites (Ladbrooks and Ashley Dene) which differ in plant available water content were established in 2014/15. In the second year of the study (2015/16), the combined effect of moisture and N was investigated. In this chapter key physiological results from the results chapters are discussed. These are then used to discuss implications for best management practices for these dryland pasture species.

7.2 Seed germination

Under decreased WP, perennial ryegrass showed the highest germination rate and final germination percentage under a wider range of temperatures compared with the other species tested (Chapter 3). For example, the optimum temperatures for germination of cocksfoot were 20 and 25 °C when moisture was non-limited. However, increasing temperature to 30 and 35 °C caused a rapid decline in germination rate of cocksfoot and tall fescue even when moisture was not limited. The HTT results suggest that, under non limiting moisture conditions, cocksfoot had the highest thermal time requirement for seed germination (114 °Cd). Furthermore, applying moisture stress (decreasing WP from 0 to -0.18, -0.37 and -0.63 MPa), especially when T>20 °C caused a more rapid decline in cocksfoot germination rate and final germination percentage than in 'Stellar AR1' perennial ryegrass, 'Finesse Q' tall fescue and 'Bareno (9045D)' brome. This suggests that under field conditions, a delay in sowing pastures in autumn, when soil temperature is high but soil moisture is low, increases the risk of failure in pasture establishment of cocksfoot more than other grasses used in this study. For example, when the WP was -0.63 MPa, which equals to 14% and 16% soil moisture for a silt loam and clay soil type, cocksfoot showed the highest germination rate and final germination percentage at 15 °C. This was close to the average top soil (10 mm) temperature at Ladbrooks (15.5 °C) and Ashley Dene (16.2 °C) for the current research (Appendices B and C). Based on the results it can be concluded that, in a dryland mixture species with faster establishment rates (perennial ryegrass), a lower soil temperature of 15 °C may benefit cocksfoot germination more than other species. However, even the maximum

germination rate of cocksfoot at its optimum temperature (15 °C) is lower than germination rate of the other species used in this study, at the same temperature.

In dryland autumn sown pastures the average soil temperature can be as high as 30 °C. At 30 °C, more than 70% of brome, perennial ryegrass and tall fescue seeds were expected to germinate once soil moisture was between 15-18% for a silt loam soil type and 18-20% for a clay soil type. At the same temperature, more than 70% perennial ryegrass and brome seeds germinated even under decreased soil moisture of 13 and 16% for silt loam and clay soil types, respectively. In contrast only 50% of cocksfoot seeds germinated at 30 °C when water was not limited and they did not germinate at 35 °C even when water was not limited so, sowing cocksfoot under these conditions in autumn is not recommended.

7.3 Pasture establishment

Under dryland conditions, it is the combined effects of moisture and temperature on germination and emergence rate, final germination/emergence percentage and seedling growth rates that should form the basis for recommendations on sowing time and compatibility of species within a mixture. The results of the current study are in line with the previous reports (Charlton and Stewart, 1999) that highlighted the rapid establishment of perennial ryegrass. Comparing the number of seedlings at both sites showed that, 62 DAS at Ashley Dene, the number of seedlings/m represented 43% to 51% of the sown seeds for brome, tall fescue and cocksfoot and 80% for perennial ryegrass. At Ladbrooks also the mean number of seedlings for perennial ryegrass was almost 20-30% higher than the other species. Under dryland conditions in this study, a combination of lower seed weight, slower germination (Figure 3-5) followed by slower seedling growth for cocksfoot resulted in the production of the smallest seedlings compared with brome and perennial ryegrass (Figure 4-5). The combined effects of these factors on establishment of cocksfoot was reflected in the lower shoot biomass of cocksfoot seedlings compared with brome and perennial ryegrass. Because of the slow establishment, in a mixture pasture cocksfoot may suffer from competition from faster growing companion species, particularly ryegrass. Therefore, only companion species with equally slow growth rate, or low rates (4-10 kg ha) of ryegrass sown or those that may provide beneficial effects such as white clover, should be considered for the sowing mixture where needed, if cocksfoot is to survive.

To have successful establishment from cocksfoot pastures, ideally, a relatively frequent lax defoliation system should be used throughout the first growing season. This is usually allow leaf expansion and photosynthesis as well as strong root system to develop. Grazing is recommended to be delayed until the following winter.

Brome seeds are larger than other species used in this study and can be expected to contain greater seed reserve. In the early stages, seedling growth is related to endosperm food reserves. Seedlings shoot dry weight for brome and perennial ryegrass were similar and higher than tall fescue and cocksfoot. The larger seedlings for perennial ryegrass was due to its faster germination/emergence while for brome this was because of the higher seed weight.

Hydrothermal time requirements for germination of tall fescue were similar to perennial ryegrass. It is likely that, the low thermal time requirements combined with a wider range of moisture stress and rapid seedling growth of ryegrass and tall fescue will favour its establishment at the expense of other species. Brock *et al*. (1982) showed a superior performance of ryegrass to 'Grasslands Roa' and a wild type of tall fescues because of much higher germination, root elongation, and tillering rates for perennial ryegrass. However, 62 DAS at Ashley Dene, root biomass and seedling numbers for 'FinesseQ' tall fescue were not different from other species. Field observations suggest that tillering rates for tall fescue was slower than the other species at Ashley Dene, and this may have caused a failure in pasture establishment for the tall fescue at Ashley Dene. The rate of tillering could be compared in future studies.

The results of the field study also showed that, tall fescue establishment was more vulnerable to invasion by weeds than the other grasses at Ashley Dene. In the establishment year (2014/15), tall fescue plots contained 23% of the weeds (mostly browntop and fathen reported in Section 4.11.6). Previous studies also reported that tall fescue is more affected by invasion of weeds such as browntop if fertility requirements are limited, as was the situation at Ashley Dene in this study (Lambert *et al*., 2004, Saul and Chapman, 2002). Therefore, for a successful establishment of tall fescue pastures, weed control is essential especially if fertility requirements are limited.

Lower PAWC caused a ≥45% lower shoot weight for brome, perennial ryegrass and tall fescue at Ashley Dene compared with Ladbrooks. Seedling shoot dry weight for cocksfoot was less than the other species in this study. However, moisture stress at Ashley Dene caused an almost 30% lower shoot weight for cocksfoot at Ashley Dene compared with Ladbrooks. Therefore, with the lower moisture content available in the Lismore stony soil at Ashley Dene, compared with the Wakanui silt loam soil at Ladbrooks, the cocksfoot seedlings root and shoot biomass were affected less than the other species. This implies that, while cocksfoot was slower to establish, in the establishment phase it would be less affected by moisture limitation compared with the other grasses used in this study. Based on the results of the current study, it can be concluded that, to have a successful establishment for dryland cocksfoot pasture, considering the narrow range of optimum temperature and moisture is necessary. However, the established seedlings would be less affected by period of moisture stress in the autumn compared with the other grasses used in this study.

7.4 Pasture production

Once pastures had established in 2015/16, annual yields ranged from 3.3-21.5 t DM/ha (Chapter 4). The lowest yield achieved from −N pastures at Ashley Dene was lower than the average expected range for dryland pastures in Canterbury. Annual DM yield for dryland cocksfoot in Canterbury was 7.6 t DM/ha compared with 4.9 t DM/ha from ryegrass pastures (Stevens *et al.*, 1992). The maximum yield determined from +N pastures at Ladbrooks and was similar to irrigated +N 'Wana' cocksfoot (Mills *et al*., 2006) and ~7 t DM/ha lower than the environmental optimum previously reported (Peri *et al*., 2002). Mean daily growth rates ranged from a minimum of 5.5-7 kg DM/ha/d in April and July 2016 for −N pastures at Ashley Dene to a maximum of 162 kg DM/ha/d for +N pastures in December and February at Ladbrooks. The maximum was higher than 130-140 kg $ha^{-1}d^{-1}$ for dryland perennial ryegrass and tall fescue in a sandy clay soil once 300 kg ha^{-1} of N was applied annually (Rinne, 1978; Lemaire *et al*., 1982). At Ladbrooks, where moisture was less limited compared with Ashley Dene, daily growth rate was minimum on June 2015 and July 2016, when low winter temperature affected pasture growth. Decreased temperatures, reduces daily rates of leaf appearance, which are usually constant in accumulated thermal time. Moreover, reduced enzyme activity which catalyses reactions related to photosynthesis may cause yield reductions under decreased temperature. In the current study, these were not measured, but there is enough evidence in the literature to support these conclusions. As an example, in wheat and barley, as temperature declined, photosynthesis reduced as a result of reduced Rubisco specificity at decreased temperatures (Bunce, 1998).

Comparison of total N recovery showed that under severe moisture stress at Ashley Dene, N recovery by the pastures was less than 30% of the N fertiliser applied. Nitrogen was not utilised by pastures at Ladbrooks in the summer when severe moisture stress suppressed pasture production. N not used by pastures may have been volatilised. Therefore, application of N under severe dry conditions is not recommended since it is not economical and not environmentally friendly.

Slower establishment of cocksfoot compared with the other grasses resulted in a higher proportion of the weeds in the establishment year (2014/15) compared with brome and perennial ryegrass. This suggests the slow establishment enabled other species to establish. If weed control was adequate then the slower establishment of cocksfoot may be beneficial for legume companion. Careful management is then required during establishment to ensure they are not suppressed. Once cocksfoot established, it withstood periods of moisture stress, and responded vigorously to autumn rain. In this study, from January to July 2016 when severe moisture stress limited production of the pastures at Ashley Dene, production of +N cocksfoot was more than other species. Application of N before 85 mm of rainfall in January 2016 caused a 50% higher DM for cocksfoot compared with the other grasses. DM production

of brome, tall fescue and perennial ryegrass did not respond to N at Ashley Dene in any regrowth cycles.

7.5 Temperature

In 2015/16, N was the most important factor affecting temperature adjusted growth rate of the pastures at Ladbrooks. Seasonal temperature variation was characterised using accumulated thermal time above the base temperature of 3 °C and the optimum temperature of 23 °C (Sections 4.9.1). +N pastures at Ladbrooks produced an average of 8.88 kg DM/°Cd/ha in Phase 1. At any given temperature, in Phase one, when moisture was less limiting compared with Phase two, +N pastures produced DM at twice the rate of the control pastures (−N). Temperature adjusted growth rate decreased to an average of 4.41 kg DM/°Cd/ha in the second phase which started in the middle of summer, once moisture stress reduced pastures production. Mills *et al*. (2006) reported lower values of 7.2 DM/°Cd/ha for irrigated +N 'Wana' cocksfoot and 7.0 DM/°Cd/ha for +N dryland when periods of moisture stress were excluded. The annual values were lower than 18.2 and 13.7 DM/°Cd/ha reported by Lemaire and Salette (1982) for +N tall fescue and cocksfoot with the same base temperature of 3 °C. However, those values were determined for the period from the last autumn defoliation to the first spring defoliation. With the onset of moisture stress in Phase 2 at Ladbrooks, N did not affect temperature adjusted growth rate. In the second phase , mean temperature adjusted growth rate for brome, cocksfoot and tall fescue was 6.5 kg DM/°Cd/ha which was higher than 0.50 kg DM/°Cd/ha for perennial ryegrass.

Under a severe moisture stress at Ashley Dene in 2015/16, N did not affect temperature adjusted growth rate of brome, perennial ryegrass and tall fescue. Due to a more severe and longer period of moisture stress at Ashley Dene, accumulated DM against accumulated thermal time was broken in to five phases. Cocksfoot pastures showed higher accumulated DM against accumulated thermal time in three phases. Only in Phase 3, N increased temperature adjusted growth rate of cocksfoot pastures.

The results both at Ladbrooks and Ashley Dene supported the previous studies showed that, under dryland conditions a minimum amount of moisture is required for pastures to be able to uptake N. The minimum amount of soil moisture required for N uptake might be different among species. For example, at Ashley Dene, 85 mm of moisture was sufficient for cocksfoot to uptake N in Phase 3 but not for the other species.

7.6 Moisture availability

In this study, the major difference between the two sites, was the different soil types of Wakanui silt loam at Ladbrooks and Lismore stony silt loam soil at Ashley Dene. Their different PAWC, resulted in different yield potentials. PAWC at Ladbrooks, as 40-65%, higher than the shallow Lismore stony soil at Ashley Dene.

+N pastures at Ladbrooks showed a 6% higher water use and a 41% higher water use efficiency compared with the control (−N) and this did not differ among species. This suggests that, differences between + and −N treatments in pasture production at Ladbrooks were mostly due to the efficiency with which that moisture was used by +N pastures. Mills (2006) also showed that, annual WUE of +N cocksfoot was more than double of −N. N indirectly affects WUE because photosynthetic capacity is increased (Peri, 2002) compared with −N pastures.

At Ashley Dene, mean total water use was similar for all pastures and was 43-47% lower than Ladbrooks. Application of N at Ashley Dene only increased total WUE of cocksfoot compared with the other species. The efficiency with which the water was used by cocksfoot was intrinsically linked to the increase in radiation interception and RUE caused by N fertiliser (Chapter 6). Among all species, only the WUE of +N cocksfoot was similar to +N pastures at Ladbrooks. However, the amount of moisture used by cocksfoot pastures at Ashley Dene was 43% lower than Ladbrooks due to the lower PAWC of Ashley Dene soil. Following the alleviation of moisture stress by 85 mm of rain in January 2016 there was evidence of compensatory growth in dryland pastures at Ashley Dene, particularly +N cocksfoot pastures. This phenomenon has been stated for crops and pastures previously (Horst and Nelson, 1979; Kramer, 1983; Mills, 2006). It is possibly due to the rapid expansion of cells which were formed during the stress period but were not able to expand. It has been reported that if cells are in the differentiation zone (Alves and Setter, 2004) and have not begun rapid expansion (Durand *et al.*, 1995) they continue to expand once re-watered to achieve similar cell size to those found in irrigated plants. Plotting water use/EPT against soil moisture deficit both at Ladbrooks and Ashley Dene showed that both dryland pastures have been under stress even in winter. From 1/10/2015 to 10/4/2016, in the second year of study, comparing the amount of monthly rainfall and PET with the long term data at Ladbrooks shows that, rainfall was well under the long term data and PET was always higher than the long term data. Therefore, even in Phase 1, when temperature adjusted growth rate of the pastures at Ladbrooks was less affected by moisture stress, the average water use/EPT against soil moisture deficit was 0.75 and 0.69 for + and −N pastures, respectively. This suggests that, even when dryland pasture production was less limited by moisture stress (Phase 1), only 75% and 69% of crop demand was met by soil moisture. With the onset of moisture stress in summer, reduction in pasture production occurred once soil moisture deficit was > 140 mm for −N and > 230 mm for +N pastures at Ladbrooks. At Ashley Dene, the critical SMD was not clear due to the lack of data linking water use/EPT

against soil moisture deficit. Since the relationship between water use/EPT and soil moisture deficit both at Ladbrooks and Ashley Dene did not reach 1.0 (it reached 1.0 only on one occasion after winter rainfall at Ladbrooks, but this was insufficient to make a model), defining the critical limiting moisture deficit for pastures was not possible in this study.

7.7 Mechanisms responsible for yield reductions

In 2015/16, the annual yield difference between the sites was primarily due to a reduction in LAIadj at Ashley Dene as affected by more severe moisture stress compared with Ladbrooks. Cellular expansion is the most sensitive plant process (Hsiao, 1973) influenced by moisture deficits (Section 2.3.1). Under moisture stress, canopy expansion reduces and decreases the area available for gas exchange. As a result, canopy expansion slows down, and less PAR is intercepted due to the formation of smaller leaves and/or fewer tillers per unit area. Mills (2006) reported that, under drought stress, a reduction in the number of tillers in combination with a reduced specific green area were the most important factors that reduced intercepted PAR and therefore the production of dryland 'Wana' cocksfoot. Comparing total intercepted PAR by +N perennial ryegrass, brome and tall fescue showed 3.50, 3.99 and 4.27 times more PAR intercepted by pastures at Ladbrooks than Ashley Dene. Annual intercepted PAR by +N cocksfoot at Ladbrooks was 2.32 times more than Ashley Dene roughly at the same year (2015/16). This was mostly because at Ashley Dene cocksfoot was less affected by moisture stress and produced more DM compared with the other species. Also, after 85 mm of rainfall in January 2016, +N cocksfoot was able to expand the leaves and intercept more PAR than other species which was reflected in higher LAIadj by +N cocksfoot at Ashley Dene. The results at Ashley Dene suggest that higher production of +N cocksfoot in January was due to both more PAR intercepted by cocksfoot as well as its higher RUE.

At Ladbrooks, the mechanism which reduced −N pasture production compared with +N pastures was primarily due to the reduction in the intercepted PAR as well as RUE by −N pastures. N supply can affect plant growth and productivity by changing leaf area and photosynthetic capacity (Novoa and Loomis, 1981). N application at Labrooks caused ~40% increase in RUE and 22% rise in total PAR intercepted, which were the main factors that increased DM production of +N pastures compared with the control (−N). N deficiency can decrease cell differentiation resulting in the production of smaller leaves due to the limiting cell numbers (Novoa and Loomis, 1981a). This caused −N pastures at Ladbrooks to produce almost 50% less DM than +N pastures.

7.8 Implications for dryland farming systems

The results of this research show that, production of monocultures of brome, cocksfoot, perennial ryegrass and tall fescue will slow/cease during periods of moisture stress which are a typical of dryland

farming systems on the east coast of New Zealand. An average production of 19.8 t DM ha^{-1} yr^{-1} for +N pastures at Ladbrooks shows that brome, cocksfoot and tall fescue, which have been previously reported as drought tolerant species, are capable of high yields in dryland pastures with a moderate moisture deficit and a relatively high PAWC when N was applied.

In a dryland system which is regularly subjected to summer moisture stress, the main goal is to select the species which have smaller decreases in growth under water limitation and are able to recover after experiencing drought conditions. This study confirms the importance of cocksfoot in dry summer areas and shows that the production of cocksfoot can be improved by increased N fertility. However, for successful establishment of cocksfoot, special care will be needed at the sowing and management stages. Cocksfoot cannot be grazed during establishment in a similar manner to a ryegrass pasture. Competition from weeds must be monitored and herbicide treatments may be required to allow it and all associated legumes to be established.

Since most of the pastures in New Zealand are N deficient, N application could be useful to increase cocksfoot yield. However, the quantity of N applied in this study is not recommended for commercial use. N fixation by legumes is important in the success of pastoral agriculture in New Zealand. Legumes provide high quality feed and are able to transfer N to the companion grass through urine or decomposition of root and shoot material (Haynes, 1980). Peoples and Baldock (2001) reported 25 kg of nitrogen fixed per one tone of legume DM grown. Thus, maintenance of a high proportion of clover in the pasture will improve pasture quality. When cocksfoot was sown with legumes such as white clover, its aggressive growth habit, caused a decrease in the clover component (Moloney, 1991). This can cause pastures to become N deficient which may restrict leaf/canopy photosynthesis and pasture production (Peri *et al*., 2002a; Peri *et al*., 2002b) and low grazing preference (Edwards *et al*., 1993). It is important to identify a legume which can survive and persist in a mixture with cocksfoot to alleviate the main yield constraint of N deficiency and the associated reduction in pasture quality (Moloney 1991; Edwards *et al*., 1993). Based on the 'MaxClover' experiment by Mills *et al*. (2008), among the five clover/grass mixtures, subterranean clover (*Trifolium subterraneum* L.)/cocksfoot pasture provided the most sheep liveweight gain. Production and quality of the cocksfoot will be improved in early spring, mostly because of N returns in urine from grazing animals which select a high clover diet before it sets seed. This raises crude protein content (Section 4.11.9) which is more acceptable to grazing livestock relative to N deficient pasture with low grazing preference (Garwood and Williams, 1967; Evans, 1978; Garwood and Sinclair, 1979). Higher liveweight gain related to the selection of a high clover diet during lambing and lactation will mean lambs will reach sale weights faster and be sold prior to drought conditions which bring the used for conserved feed. It has been reported previously that the reduction in clover content over time decreases animal production (Edwards *et al*., 1993). A

legume with an annual life cycle may also advantage cocksfoot in moist summers due to the decomposition of residual root and shoot biomass which would release N to the accompanied grass.

7.9 Recommendations for future research

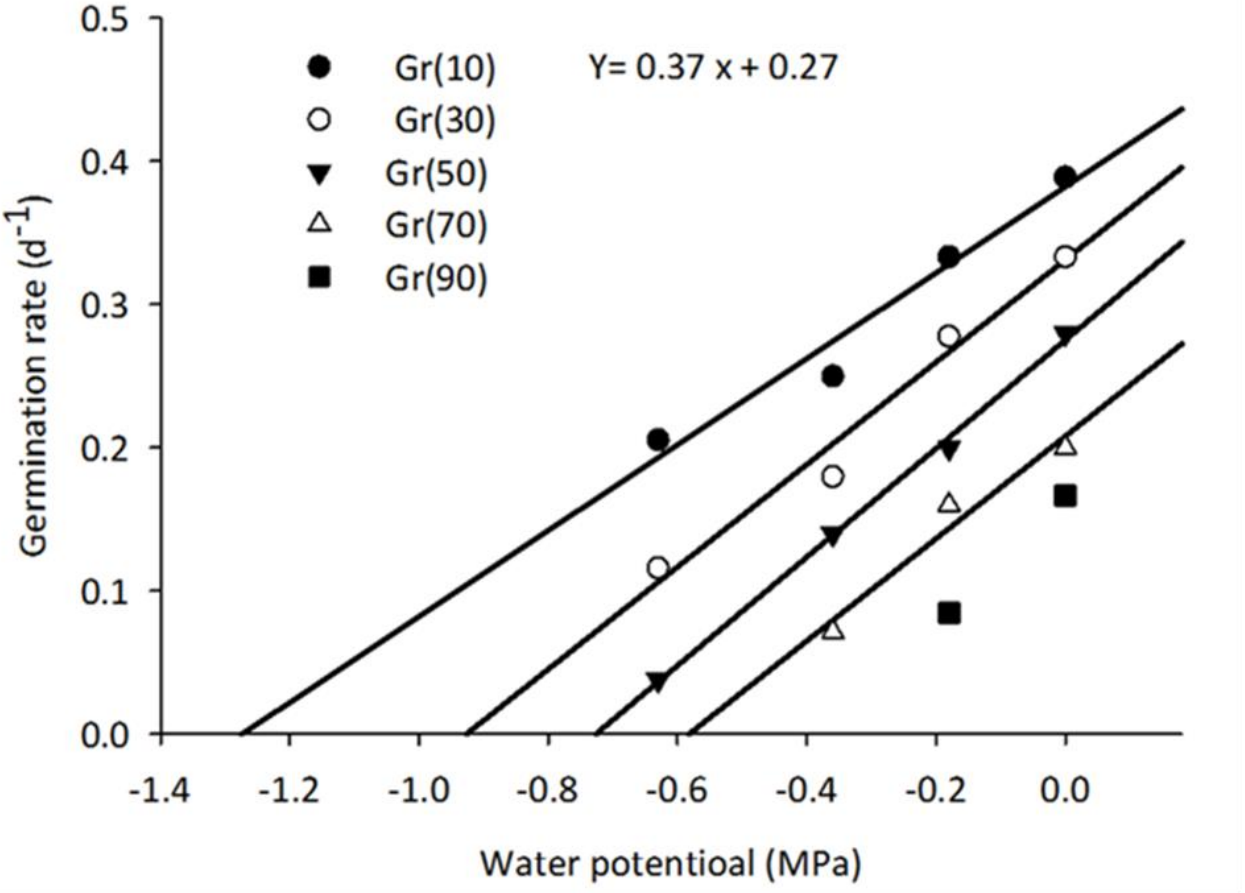
The thesis results highlighted several areas which require further clarification to complement. These include:

a. Under a severe soil moisture stress at Ashley Dene, +N cocksfoot pasture produced twice the DM compared with the other species. This was mainly due to a higher light intercepted by cocksfoot than the other species. However, it is unclear whether higher intercepted light by the cocksfoot canopy was because of greater leaf elongation or higher tiller numbers. Leaf elongation and tillering number measurements are recommended for further clarification.

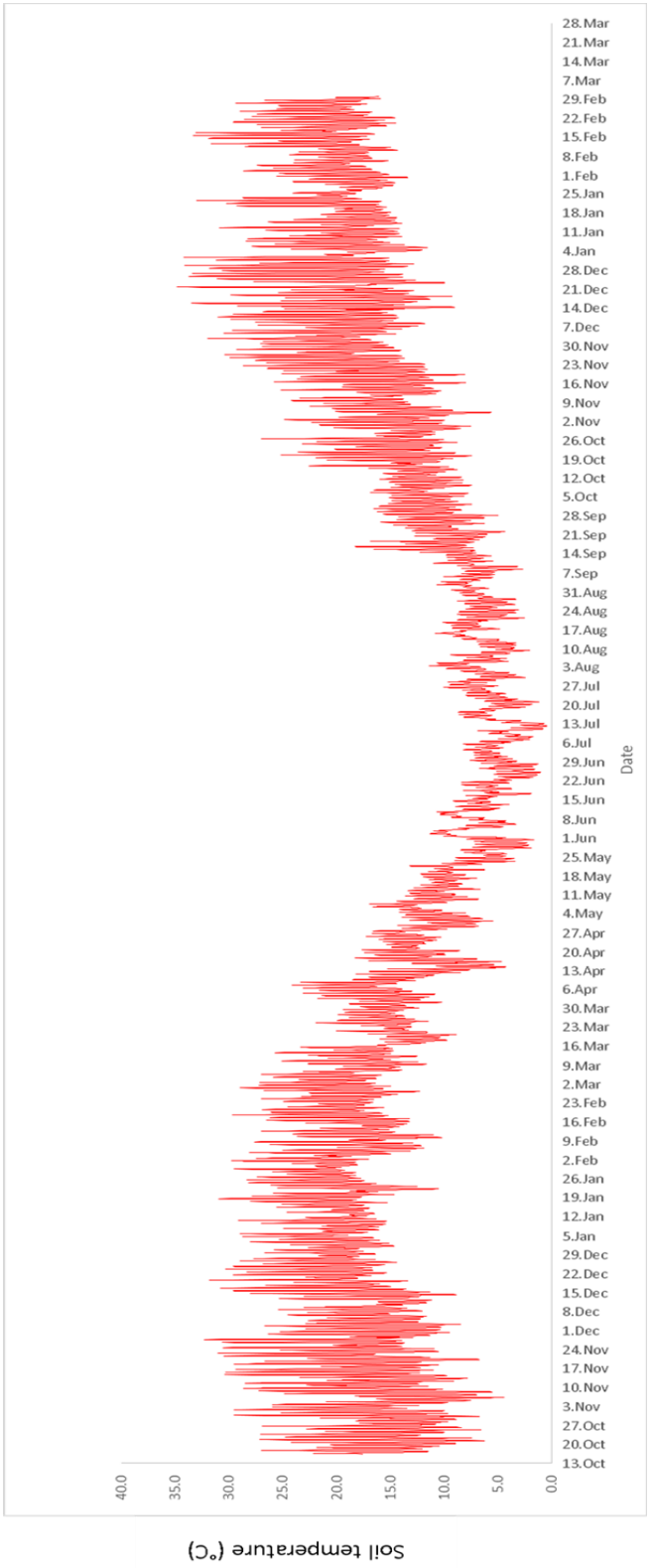
b. At Ashley Dene, tall fescue did not show an advantage over other grasses in terms of establishment. Based on visual field observations, it was mostly because of the lower tiller number per plant for tall fescue. But tiller number was not measured in this study. Further investigations are required to measure tillering number to understand the slow establishment of tall fescue at Ashley Dene.

c. This research focused on new established pastures and investigated agronomic and physiological performance of the pastures during 18 months of the study. Further research is required to evaluate the effect of different moisture levels on the persistence of these pastures. Therefore, continuing monitoring for several further seasons is recommended.

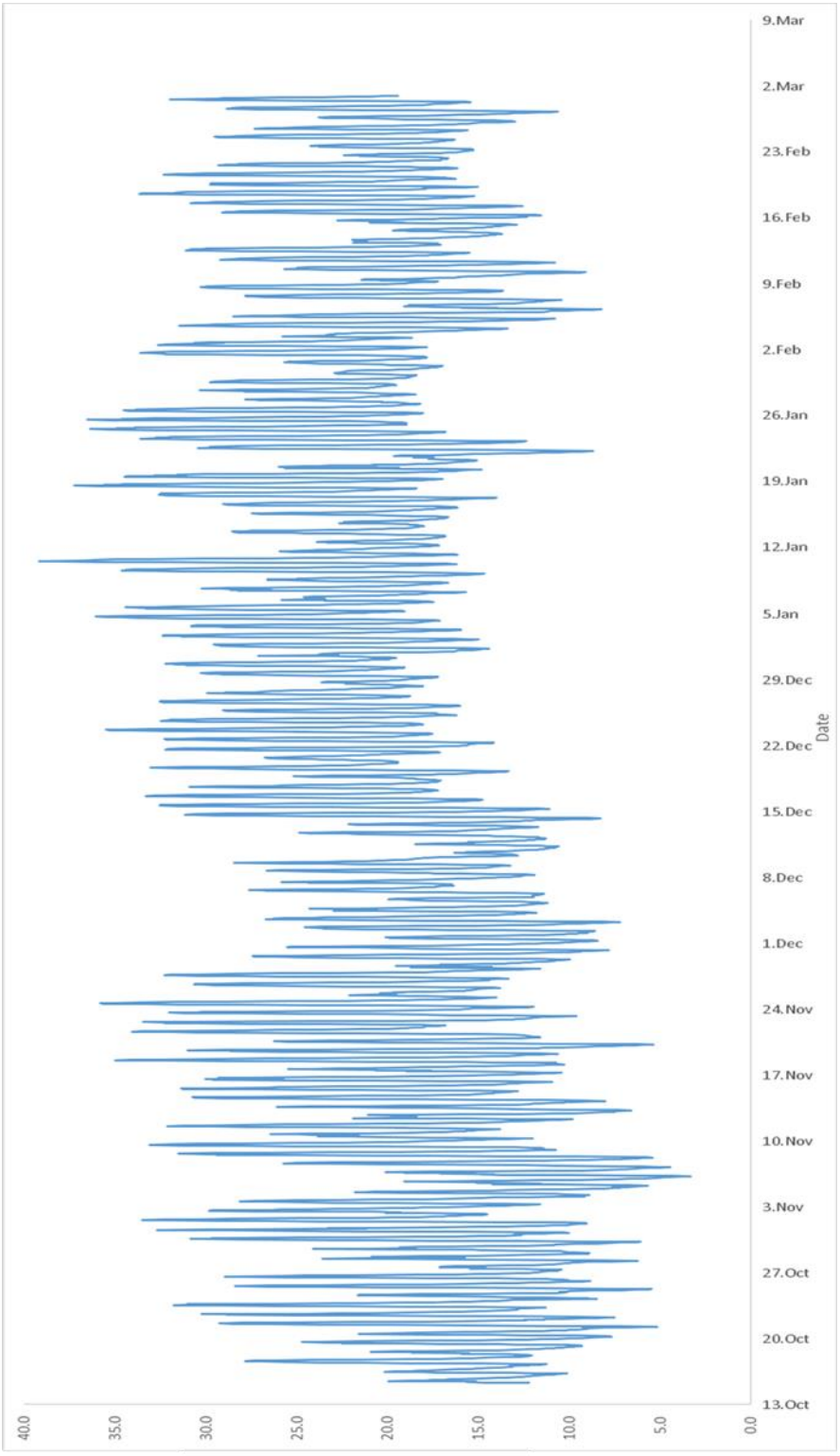
Appendix A Tall fescue germination rates (GR) for 10th – 90th percentiles versus WP when T= 20 °C.



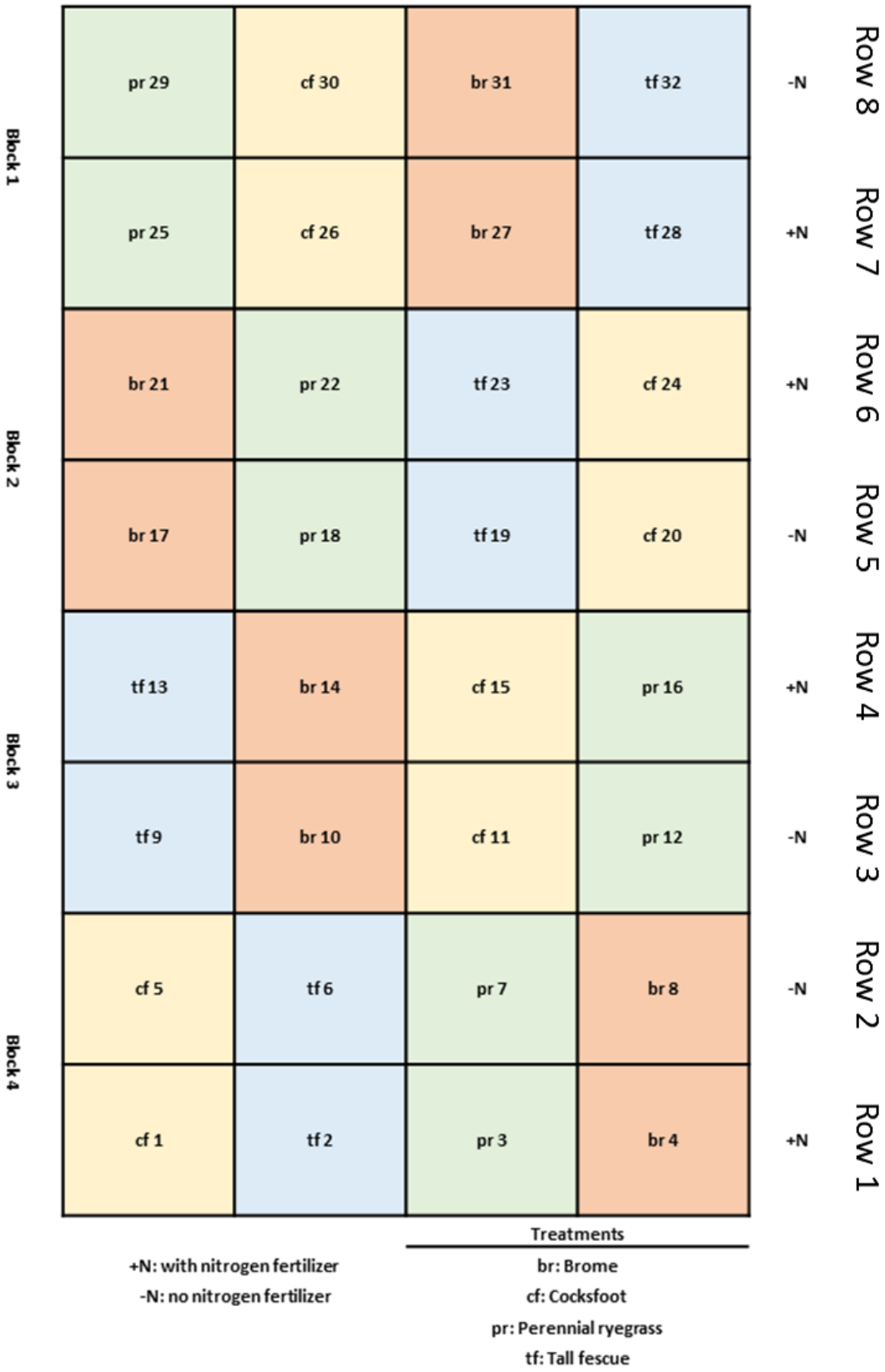
Appendix B Hourly soil temperature (°C) at the depth of 10 mm, from 15/10/2014 to 29/2/2015 at Ladbrooks.



Appendix C Hourly soil temperature (°C) at the depth of 10 mm, from 15/10/2014-29/2/2015 at Ashley Dene.



Appendix D Experimental plan at Ladbrooks and Ashley Dene C8, in 2015/16.



Appendix E

Mowing time and date, growth/ regrowth period (day) at Ladbrooks. Grazing time and date, growth/ regrowth period (day), number of ewes and duration of grazing (day) at Ashley Dene (2015/16).

Site	Mowing order	Mowing date	Regrowth period (day)	Site	Grazing order	Grazing date	regrowth period (day)	Number of ewes	Grazing period (day)
Ladbrooks	1	4/4/2015	170	**Ashley Dene**	1	14/7/2015	272	20±5	8
Ladbrooks	2	16/6/2015	73	**Ashley Dene**	2	30/9/2015	78	80±5	2
Ladbrooks	3	18/9/2015	94	**Ashley Dene**	4	1/11/2015	87	80±5	2
Ladbrooks	4	15/10/2015	27	**Ashley Dene**	5	21/1/2016	113	80±5	3
Ladbrooks	5	11/11/2015	27	**Ashley Dene**	6	23/4/2016	93	80±5	3
Ladbrooks	6	10/12/2015	29						
Ladbrooks	7	13/1//2016	33						
Ladbrooks	8	11/2/2016	28						
Ladbrooks	9	14/3/2016	32						
Ladbrooks	10	23/4/2016	37						
Ladbrooks	11	10/6/2016	78						

Appendix F Application date and rate of nitrogen fertiliser (urea (46,0,0,0)) at Ladbrooks and Ashley Dene.

Date	Site	Fertiliser	Plots	Rate (kg N ha^{-1})
18/9/2015	Ladbrooks	urea (46,0,0,0)	+N plots except plots 14, 15, 18, 19, 22 and 23	100
18/9/2015	Ladbrooks	urea (46,0,0,0)	Plots 14, 15, 18, 19, 22 and 23	50
21/9/2015	Ashley Dene	urea (46,0,0,0)	+N plots	100
15/10/2015	Ladbrooks	urea (46,0,0,0)	+N plots	200
11/11/2015	Ashley Dene Ladbrooks	urea (46,0,0,0)	+N plots	100
16/12/2015	Ladbrooks Ashley Dene	urea (46,0,0,0)	+N plots	100
13/1/2016	Ladbrooks	urea (46,0,0,0)	+N plots	100
8/2/2016	Ashley Dene	urea (46,0,0,0)	+N plots	100
11/2/2016	Ladbrooks	urea (46,0,0,0)	+N plots	100
14/3/2016	Ladbrooks	urea (46,0,0,0)	+N plots	100
20/4/2016	Ladbrooks	urea (46,0,0,0)	+N plots	100
22/4/2016	Ashley Dene	urea (46,0,0,0)	+N plots	100

Note: Based on soil test results, in the first application of N fertiliser, 50 kg N ha^{-1} was applied to the +N plots with N background.

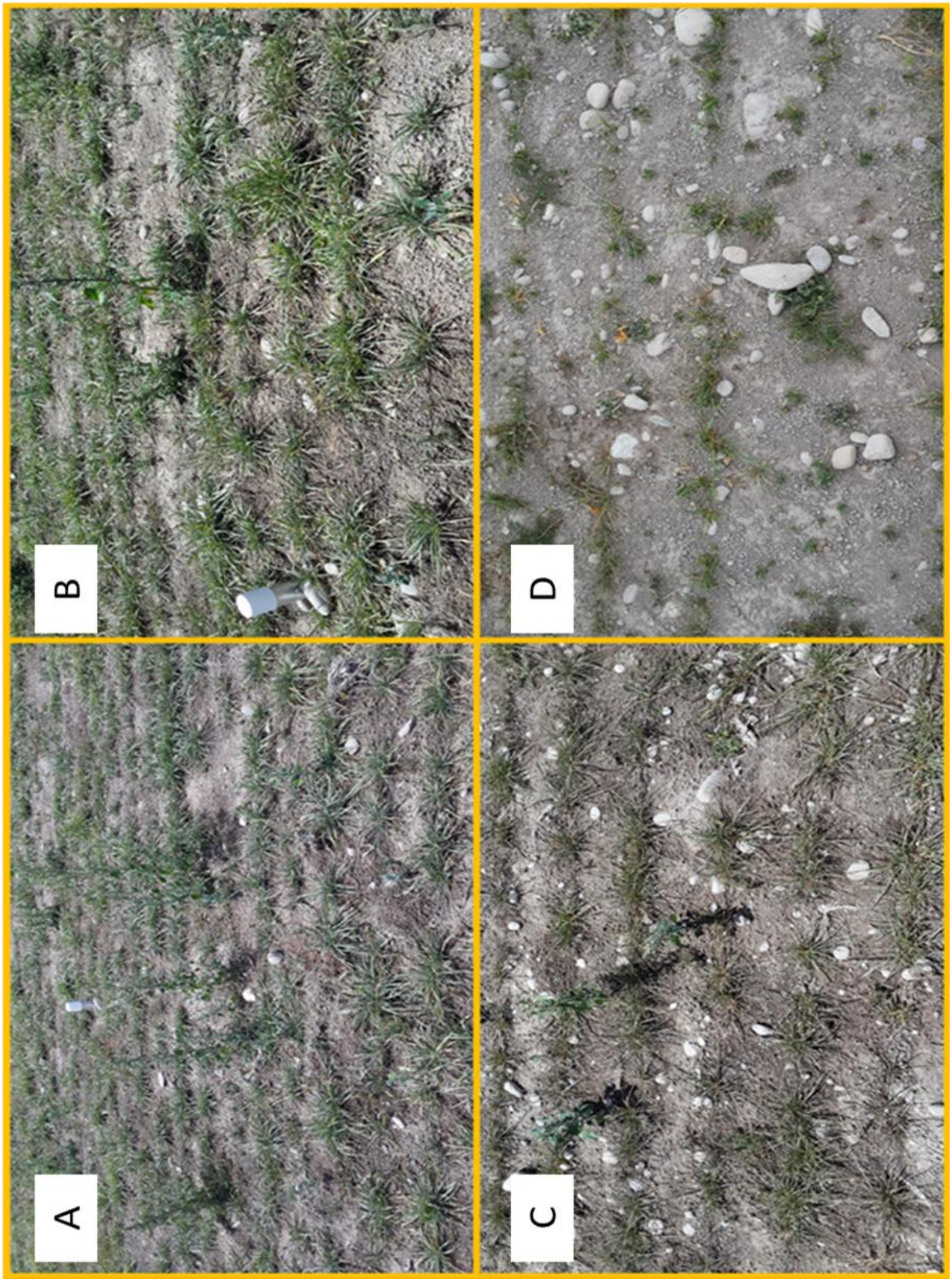
Appendix G DM production (kg ha^{-1}) of individual regrowth cycles in 2014/15 and 2015/16 for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown at Ladbrooks, Canterbury, New Zealand. Treatments are + nitrogen (+N) and – nitrogen (–N).

	+N				-N													
Date	Br	Cf	Pr	Tf	Br	Cf	Pr	Tf	Species significance	LSD (P<0.05)	e.s.e	Nitrogen Significance	LSD (P<0.05)	e.s.e	Species* Nitrogen Significance	LSD (P<0.05)	e.s.e	Comments
7/01/2015	530	618	542	660	487	629	815	709	0.393	-	80	-	-	-	-	-	-	Latin square
2/02/2015	993	775	1102	975	821	1256	1966	1125	0.105	-	185	-	-	-	-	-	-	Latin square
24/02/2015	1680	958	1509	1301	1540	1267	1660	1992	0.269	-	214	-	-	-	-	-	-	Latin square
31/03/2015	1444	1784	2211	2147	1956	1858	1569	2106	0.598	-	225	-	-	-	-	-	-	Latin square
24/04/2015	973	534	895	769	849	465	755	785	<.001	157	54	-	-	-	-	-	-	Latin square
9/06/2015	2079	1614	1485	1400	2048	1577	1926	1464	0.008	352	121	-	-	-	-	-	-	Latin square
12/08/2018	1635	1191	1752	1448	1405	1200	1053	1067	0.605	-	184	-	-	-	-	-	-	Latin square
11/09/2015	1231	1758	1858	1599	1295	1510	1464	1155	0.103	-	129	-	-	-	-	-	-	Latin square
8/12/2015	2232	2306	3355	2391	1091	1364	1550	1511	0.073	600	188	<.001	271	88.1	0.079	674	225	Strip plot
7/01/2016	4461	4471	4056	4570	1540	1986	1835	1884	0.613	599	187	<.001	659	214	0.865	1052	356	Strip plot
9/02/2016	4023	4118	4375	3773	970	1253	1774	1145	0.119	562	176	<.001	174	56.5	0.204	591	193	Strip plot
14/03/2016	2605	2187	2187	2609	1105	1025	792	1108	0.042	291	90.8	<.001	391	127	0.896	600	201	Strip plot
20/04/2016	2063	1872	2056	2577	760	1154	1385	724	0.501	480	150	<.001	293	95.1	0.028	595	202	Strip plot
10/07/2016	2014	1456	1410	1422	607	483	1016	370	0.357	569	178	<.001	372	120.7	0.262	726	246	Strip plot

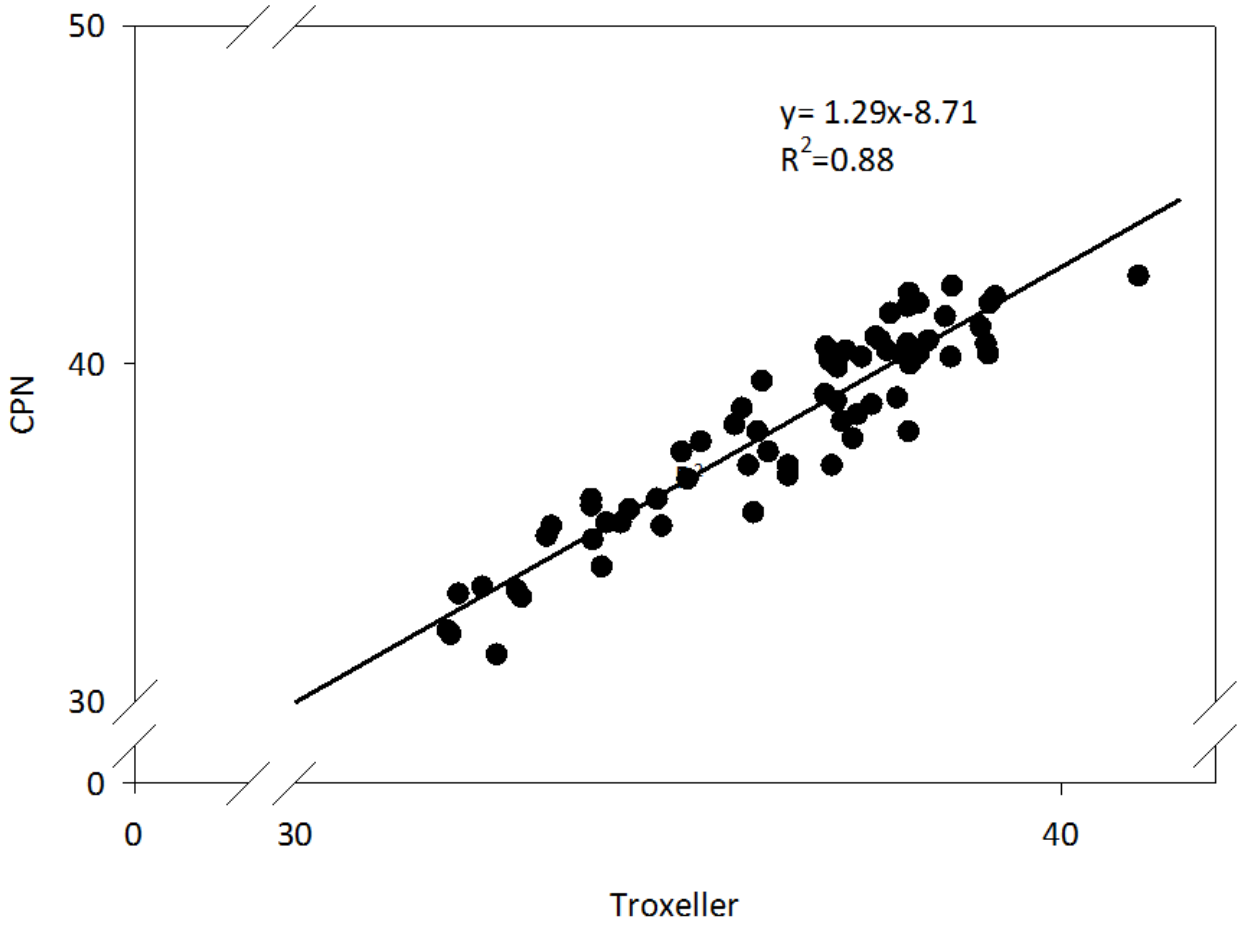
Appendix H DM production (kg ha^{-1}) of individual regrowth cycles in 2014/15 and 2015/16 for monocultures of brome, cocksfoot, perennial ryegrass and tall fescue grown at Ashley Dene, Canterbury, New Zealand. Treatments are + nitrogen (+N) and – nitrogen (−N).

	+N					-N					Species significance	LSD		Nitrogen Significance P	LSD		Species*Nitrogen Significance P	LSD		
Date	Br	Cf	Pr	Tf	Br	Cf	Pr	Tf	P value	(P<0.05)	SEM	value	(P<0.05)	SEM	value	(P<0.05)	SEM	Comments
7/01/2015	475	448	376	392	411	600	372	447	0.66	-	-	-	-	-	-	-	-	Latin square
17/04/2015	599	600	386	379	464	551	483	453	0.37	-	-	-	-	-	-	-	-	Latin square
7/07/2015	1822	1344	1527	1158	1711	1418	1673	1246	0.015	0.35	0.12	-	-	-	-	-	-	Latin square
26/09/2015	1115	1100	1948	1460	1061	962	1395	1302	0.045	0.49	0.17	-	-	-	-	-	-	Latin square
27/10/2015	100	489	636	114	165	161	540	209	0.006	0.27	0.09	-	-	-	-	-	-	Latin square
20/01/2016	1043	1374	723	621	829	1003	828	834	0.002	0.20	0.06	0.428	-	-	0.084	-	-	Strip plot
21/04/2016	683	1290	874	555	446	625	583	545	<.001	0.07	0.02	0.001	0.16	0.05	0.052	0.23	0.07	Strip plot
7/07/2016	1822	1344	1527	1158	1711	1418	1673	1246	0.038	0.38	0.01	0.723	-	-	0.917	-	-	Strip plot

Appendix I Brome (A), cocksfoot (B), perennial ryegrass (C) and tall fescue (D) at Ashley Dene C8 experimental site on 20/1/2015.



Appendix J Cross calibration data measured to calibrate CPN based on Troxeller neutron probe data.



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